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THE DETERMINATION OF MERCURY IN ENVIRONMENTAL SAMPLES

Laboratory
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SEP 1974

ENVIRONMENTAL BRANCH



Ministry
of the
Environment

The Honourable
William G. Newman,
Minister

Everett Biggs,
Deputy Minister

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THE DETERMINATION OF MERCURY IN ENVIRONMENTAL SAMPLES

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Environmental
Trace Contaminants
Section

RECEIVED

SEP 2 1975

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Ministry
of the
Environment

THE DETERMINATION OF MERCURY
IN ENVIRONMENTAL SAMPLES

Mercury is geologically a rare element and comprises less than 3×10^{-6} per cent of the earth's crust. There are comparatively few locations on the earth's surface where mercury is present in more than trace amounts. Most of the element occurring naturally is in the form of the highly insoluble mercuric sulfide, the principle component of cinnabar.

The industrial use of mercury is quite widespread, ranging from small scale uses such as thermometers and electrical switches to the large amounts used in the manufacture of caustic soda and chlorine in the chlor-alkali industry. Mercury compounds are used quite extensively as biocides, fungicides, and in the manufacture of pharmaceuticals and paints.

The recent concern for mercury pollution began with the Minimata case where hundreds of Japanese were poisoned, resulting in at least fifty fatalities. (1). This was followed by a similar outbreak of poisoning in Niigata, Japan, (2) and in the mid-sixties by reports from Sweden of high levels of mercury in fish and wildlife (3). As a result of these studies, it was shown that any form of mercury entering an aquatic system could be converted (by micro-organisms and biochemically derived alkylating systems) to methyl mercury derivatives (4). These compounds follow a process of concentration as progressively larger animals feed on smaller ones, until the concentration endangers higher forms of life. Due to the low concentration of mercury that can cause problems and to the various matrix effects which have to be considered, the analytical work associated with this problem is quite complex.

1. Sample Handling and Preservation

A: BIOLOGICAL MATERIAL

When biological material is collected for mercury analysis, it should be stored in a plastic bag or other non-metal container to prevent cross-contamination and should be frozen immediately to retain moisture and retard protein breakdown.

B: WATER SAMPLES

Samples should be taken in glass containers and not allowed to come into contact with any metal objects. (Cap liners, sampling vessels, etc.). It has been established

that losses of mercury occur when unpreserved samples are stored in polyethylene or glass containers (5) (6) (7) (8). These losses appear to result from inorganic mercury adsorption on the walls of the container.

For total mercury analysis, glass bottles with Teflon cap liners should be used. The samples should be preserved with potassium permanganate and nitric acid immediately after sampling. The *pH* must be below 1 and enough potassium permanganate should be present to maintain a pink colour (8).

Storage of samples for more than *three* days can result in a considerable loss of mercury even with preservation, so minimum time should elapse between sampling and analysis of samples.

C: SEDIMENT

Care should be taken when sampling to ensure that samples are representative of the area being examined. Samples should be transported directly to the laboratory in glass jars with Teflon-lined caps. Sediments from different sampling points should be kept separate to avoid cross-contamination. Sampling devices and containers must be free of mercury contamination and should be designed so as to minimize the chances of mercury loss by amalgamation.

2. Selection of Method

A: GENERAL TECHNIQUES

There are several different methods used for the analysis of mercury.

Colorimetry - Dithizone Extraction

This method requires a preliminary digestion step to destroy all organic matter present and it is cumbersome, slow and subject to interferences (*Cu, Ag, Al, Fe, Ni, etc.*) (9-11).

Neutron Activation

This method is impractical for most laboratories due to the high cost of the equipment and the length of time required for results. Chemical treatment and separation is normally required for sensitive, reliable analyses (12, 13).

Atomic Absorption Spectrophotometry - Flame

The shortcomings of the dithizone extraction procedure made it imperative that a more selective method be found. The conventional AAS procedure, while selective, lacks sensitivity despite the use of enhancing techniques (14), and consequently this method is not widely used.

Flameless Atomic Absorption Spectrometry

The FAAS technique is sensitive, rapid, reproducible, and relatively devoid of interferences. It can be applied to a wide variety of sample types, provided adequate pretreatment measures are followed (15-18).

Methyl Mercury by Gas-Liquid Chromatography

The Gas-liquid chromatographic method is used to determine methyl mercury in environmental samples and is the method used in most laboratories involved in mercury analyses.

B: TECHNIQUES APPLIED IN MINISTRY LABORATORY

TOTAL MERCURY

Biological Material

Several different acids and combination of acids, as well as the use of alkalies and enzymatic means of tissue breakdown were investigated for the digestion of biological matter. As a result of these investigations, two techniques, both based on acid-permanganate digestion were found to be rapid and reliable. One method is based on heating the acidified samples in a shaking water bath; the other uses a heated aluminum block as a heat source. The latter method is the method presently in use. Total mercury is measured in the digestate by FAAS in both cases.

Water (Natural Sources) Method A

Many colorimetric procedures for the determination of mercury in waters have been published (19-21) but these methods have not been applied in the M.O.E. laboratory since they are too time-consuming, insufficiently sensitive and susceptible to interferences.

The Flameless Atomic Absorption technique is used as the standard procedure. The method is based on a published E.P.A. procedure (22).

Method A is designed for the analysis of water containing low levels of mercury (less than 10 ng/ml). This technique makes use of the Laboratory Data Control Mercury Monitor and highly purified reagents. The digestion procedure used in this method is not capable of eliminating excessively high amounts of organic matter or chlorides, and when such compounds are expected to be present, an alternative but less sensitive method (Method B) is used.

Water (Industrial Sources) Method B

Method B is designed for the analysis of aqueous samples that are not amenable to analysis using Method A. These samples are usually industrial effluents or in-plant liquids that are suspected of having high mercury content (in the $\mu\text{g/ml}$ range and higher) or containing excessive amounts of chemicals capable of interfering with Method A. The digestion described in the following procedure usually eliminates problems from these inter-ferents, but because the digestion requires considerable volumes of reagents the blank increases, thereby diminishing the sensitivity.

Sediments

Several different acids and combinations of acids as well as some extraction techniques were investigated for the analysis of total mercury in sediment samples. The acid leaching technique employed is efficient and when combined with the FAAS technique provides the required precision, sensitivity and accuracy.

Combustion-Amalgamation Technique

The advantage of the combustion-amalgamation technique in the measurement of mercury in various types of samples (i.e. sediments, biological material) lies in the fact that a time-consuming digestion procedure is not required and this eliminates errors caused by sample or reagent contamination, and the length of time required for an individual analysis is greatly reduced.

METHYL MERCURY

Fish Tissue

Gas chromatography with an electron capture detector is used to measure methyl mercury, after a selective extraction is performed. Although samples require complex individual treatment, the method is accurate and sensitive, and is specific for methyl mercury. The method is a modification of Westoo's procedure (23, 24, 25, 26).

THE DETERMINATION OF TOTAL MERCURY
IN BIOLOGICAL MATERIAL
Water Bath Heating, FAAS Measurement

SUMMARY

Substance determined.	Total mercury.
Interpretation of results.	The total mercury concentration in biological material is the sum of all forms of mercury present in the sample. Total mercury in fish is generally interpreted as being indicative of the methyl mercury content, since methyl mercury is usually 85-100% of the total mercury concentration.
Principle of method.	The biological material is digested in a mixture of sulfuric and nitric acid, then oxidized with potassium permanganate, and the excess permanganate is reduced with hydroxylamine sulphate, and the divalent mercury is reduced with stannous chloride. The vessel containing the sample is attached to the FAAS system, and the sample is aerated. The mercury now present as the atomic form Hg^0 is purged through the system. The Hg^0 atoms absorb energy at the resonance wavelength for mercury and this absorbance is measured spectrophotometrically.
Time required for analysis.	A single analysis requires about 1.5 days, from the initial weighing to calculations. Batch testing can result in approximately forty analysis in duplicate in 1.5 days.
Range of Application	Beer's Law is obeyed over the range 0.01 to 1.0 ug. Since 1.0 ug is approximately 90% full scale, sample aliquots containing more than 1.0 ug must be diluted.
Precision	1.95 ± 0.08 ug/g for ten determinations performed on a gelatin standard containing 2.00 ug/g mercury.
Accuracy	From the precision data above, $97.5 \pm 4\%$.
Limit of Detection	About 0.02 ug/g, based on the normal weight of sample taken (0.5 g). It is likely that a lower detection limit would be achieved by taking larger samples.
Interferences and short-comings.	There are relatively few compounds that will interfere at the levels present in biological material. Of concern are chlorides, and oxides

SUMMARY

of nitrogen but a proper digestion procedure will eliminate these problems. Water vapour is eliminated by a water trap and by the use of radiant heat from a tungsten bulb.

Volatile aromatics and some diatomic gases can absorb at the mercury resonance wavelength, but these are normally absent after the digestion step.

Minimum
sample.

A minimum of 5 grams of homogenized material is desirable.

Preservation
and sample
container

Biological samples should be quick frozen and placed in a clean, mercury-free container until analyzed.

Safety
considerations

Safe handling of all acids must be observed, and care should be taken when biological matter is being processed to prevent possible infection.

THE DETERMINATION OF TOTAL MERCURY

IN BIOLOGICAL MATERIAL

Water Bath Heating, FAAS Measurement

1. Introduction

Frozen samples are thawed and homogenized again with a glass stirring rod to redistribute any liquid that could partition out during thawing. Approximately 0.3 to 0.5 gms of homogenized tissue are accurately weighed into 30 ml micro-Kjeldahl tubes. Five ml of a 4:1 volumetric ratio of sulfuric-nitric acids are added to the tissue and the tubes are placed in an oscillating water bath at 80°C for four hours. The digestate is allowed to cool and saturated potassium permanganate solution is then added as a further oxidant and the samples are heated for an additional 1.5 hours. The samples are then allowed to stand overnight at room temperature. Hydroxylamine sulfate solution is added to reduce any excess permanganate or manganese dioxide. Immediately prior to purging the sample with air, stannous chloride is added to reduce the mercury to its atomic state. The mercury vapour is then passed through the FAAS system and the resultant absorption is measured spectrophotometrically and presented on a recorder.

2. Interferences

There are a number of elements or their oxides that can interfere both chemically and/or spectrally with mercury when analyzed using FAAS. Practically none of these are present in biological material at sufficient levels to cause problems.

Selenium, tellurium, antimony, bismuth and arsenic are reported to interfere chemically, but this effect has not been observed in biological samples in this laboratory.

A group of interfering substances that cause problems because of their positive spectral interference are water vapour, NO_2 , Cl_2 and any volatile organic solvents that absorb at 253.7 nm.

Water vapour is eliminated from the system by the insertion of a coil in an ice bath followed immediately by a water trap prior to the optical cell. As a further precaution a tungsten element lamp shines on the cell to maintain its temperature about 10°C above ambient, thereby preventing condensation of water vapour on the cell windows.

NO_2 , CO_2 and Cl_2 should not be present after the routine digestion step.

ORGANIC SOLVENTS (ACETONE, CHLOROFORM, ETC.) SHOULD NOT BE USED IN THE LABORATORY OR FOR CLEANING GLASSWARE BECAUSE THEY CAN FORM VAPORS WHICH INTERFERE WITH THE MERCURY MEASUREMENT.

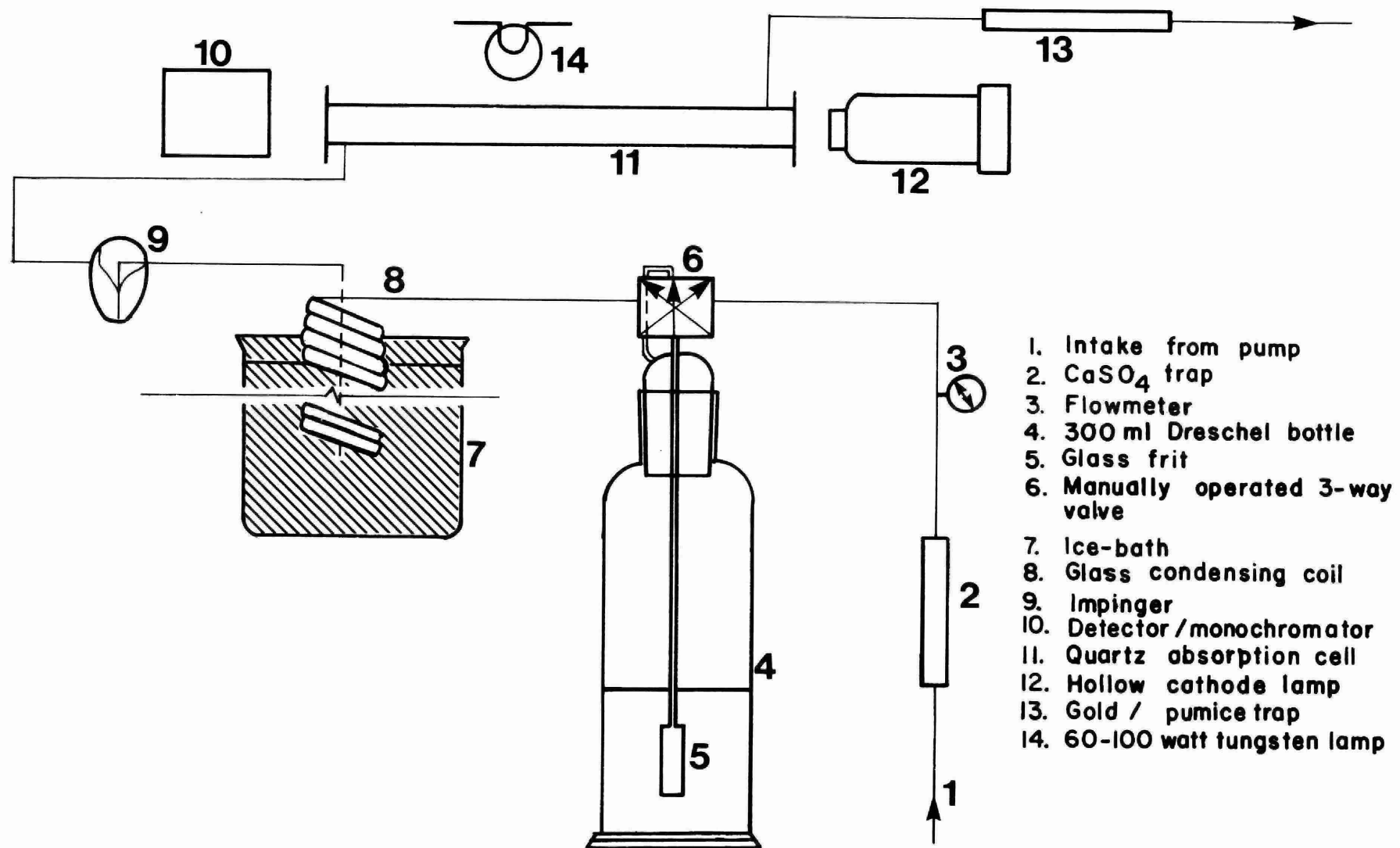
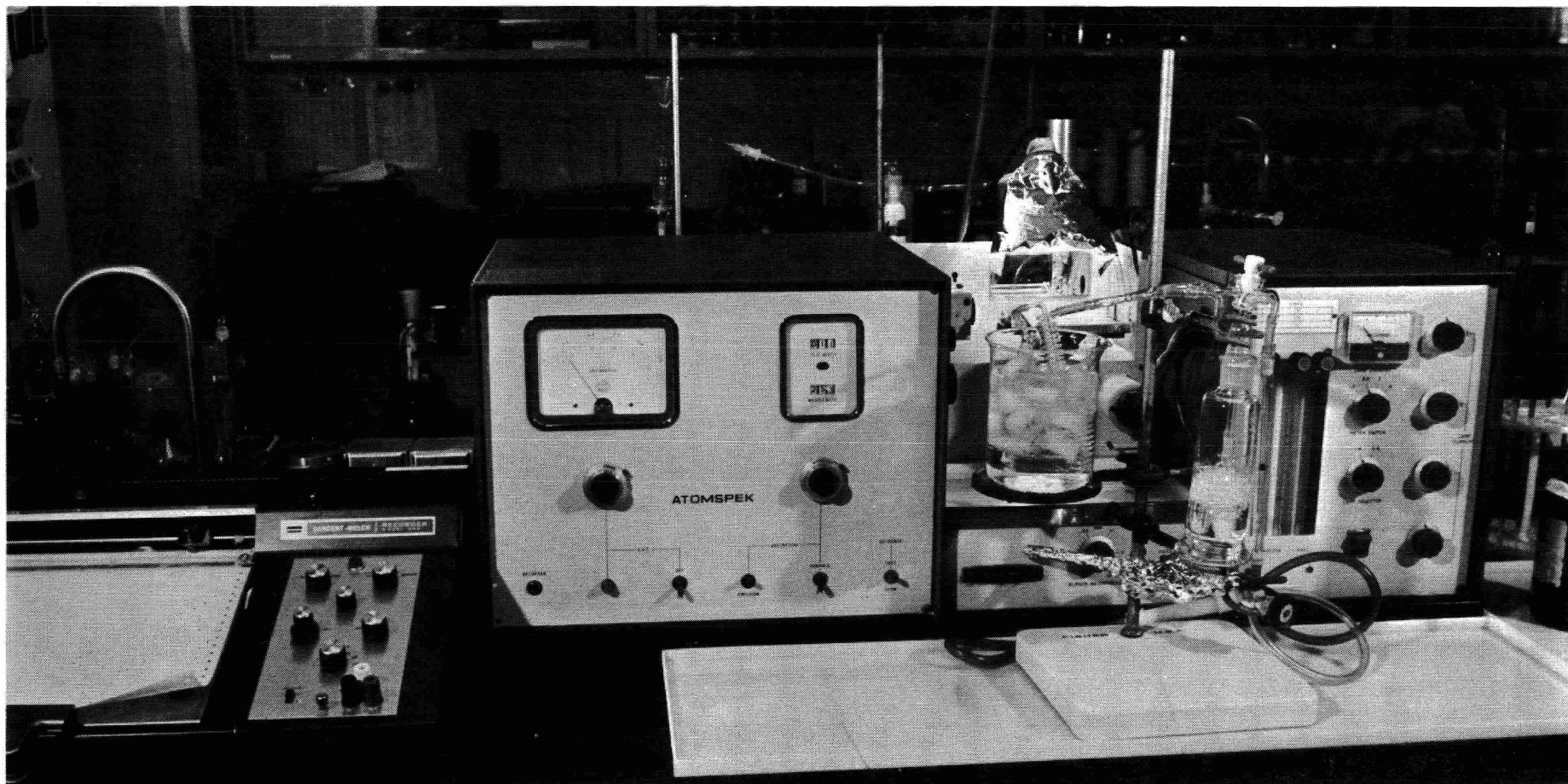


Diagram 1

Faas Determination Apparatus



**Plate I Atomic absorption spectrophotometer converted to
flameless operation**

3. Apparatus

REFER TO DIAGRAM 1 AND PLATE 1 FOR A PICTORIAL VIEW OF THE APPARATUS.

- a) Hilger-Watts Atomspek Atomic Absorption Spectrophotometer.
- b) Sargent Welch recorder; (model SRG).
- c) Optical cell; 18 cm long with a 1.5 cm diameter and quartz windows.
- d) Drying tube; plastic 11.5 cm x 2.0 cm diameter filled with calcium sulfate. Replace daily.
- e) Hollow Cathode Lamp; mercury cathode, ASL.
- f) Analytical balance; single pan (Mettler).
- g) Dreschel bottle; 250 ml volume, Quickfit (BS2461) with a 24/29 ground joint.
- h) Plunger device; composed of two parts -
 - (1). Pyrex tubing, I.D. 9 mm; O.D. 11 mm, and 20 cm in length.
 - (2). Glass rod 8 mm in diameter, 25 cm in length.
- i) Mixing coil; as supplied for Technicon auto-analyzer, (13 cm long).
- j) Tungsten element bulb; (100 watt), and a retort stand for support; used to heat optical cell.
- k) Flowmeter, Gilmont (size #3).
- l) Kjeldahl flask, micro; 30 ml volume, round bottom, long neck, Kimax glass.
- m) Water bath shaker, Eberbach (#6250) capable of maintaining 80°C.
- n) Flask holders; Eberbach (#6090).
- o) Gas dispersion tube; with fritted glass cylinder, 250 mm length, stem diameter 8 mm.
- p) Teflon stopcock; three way, Quickfit 24/29 used for directing flow of air (1) bypassing sample and (2) through sample, thus aerating it.
- q) Gas bubble counter; (Canlab #C7778-C) used as water droplet trap.

4) Compressed air.

IF A PUMP IS USED THEN NO ORGANIC VAPOURS SHOULD BE ALLOWED TO PERMEATE THE ATMOSPHERE OF THE LABORATORY.

ALL GLASSWARE AND APPARATUS MUST BE THOROUGHLY CLEANED PRIOR TO USE.

Cleaning of the Apparatus

- a) The optical cell should be cleaned once a week and/or following contamination with excessively high level mercury samples. Rinse cell with
- (1) water
 - (2) nitric acid
 - (3) methanol

The cell should then be blown dry with air.

- b) The drying tube should be refilled with fresh calcium sulfate daily and the tube purged with air to remove dust particles before attaching to the system.
- c) The teflon stopcock should be cleaned every week (minimum) with chromic acid, water, methanol and then air-dried.
- d) All polyethylene and glass tubing should either be replaced or rinsed with water, followed by methanol and blown dry with a stream of air.
- e) The dreschel bottle should be cleaned thoroughly every day or after an extremely high sample, with a cleaning solution containing 1% EDTA in 20% NaOH. Bottles are usually soaked in this solution for twelve hours.

ONLY ONE BOTTLE IS USED FOR STANDARDS AND SAMPLES DURING A RUN BECAUSE THERE IS A "BOTTLE EFFECT" WHICH CAUSES SLIGHT BUT SIGNIFICANT DIFFERENCES IN READINGS FROM BOTTLE TO BOTTLE.

- f) Kjeldahl flasks are cleaned immediately after use with
- (1) soap and water
 - (2) chromic acid
 - (3) distilled water
- Flasks must be dry before re-use.

4. Reagents

ALL REAGENTS SHOULD BE ANALYZED IN THE LABORATORY AND ONLY THOSE WITH A SUFFICIENTLY LOW MERCURY CONTENT SHOULD BE USED.

- a) Sulfuric acid; reagent grade, concentrated.
Experience with a variety of acids indicates that either Baker Analyzed or McArthur Chemicals sulfuric acids are acceptable. Reagents other than those mentioned in this section may possess the required degree of purity.
- b) Nitric acid; reagent grade, concentrated.
Experience with a variety of acids indicates that Baker Analyzed reagent is satisfactory.
- c) Hydrochloric acid; reagent grade, concentrated.
Mallinckrodt reagent has been found satisfactory.
- d) Potassium permanganate; analytical reagent.
BDH Analar crystals have been found satisfactory.
- e) Hydroxylamine sulfate; laboratory reagent.
BDH reagent is satisfactory.
- f) Stannous chloride; analytical reagent.
BDH Analar reagent is satisfactory.
- g) Sodium Hydroxide; Certified A.C.S. Electrolytic Pellets.
- h) Ethylenediaminetetra-acetic acid; laboratory reagent.
BDH disodium salt is satisfactory.
- i) Mercuric chloride; reagent grade.
As supplied by Mallinckrodt Chemicals.
- j) Methyl Mercuric Chloride; reagent grade.
Alfa Organics is a satisfactory supplier.
- k) Digestion Acid; The mixture contains a volumetric ratio of four parts sulfuric acid (concentrated) to one part nitric acid (concentrated).

MIXING CAUSES AN EXOTHERMIC REACTION: ACCORDINGLY, EXTREME CARE SHOULD BE EXERCISED DURING THIS STEP.

- l) Potassium Permanganate Solution; The solution should be saturated (60 g/l) at room temperature, then allowed to stand for at least 48 hours in sunlight before use, to form small amounts of MnO_2 , which slowly settles out. This step greatly reduces the risk of mercury contamination from the $KMnO_4$.

- m) Hydroxylamine Sulfate solution; The solution (20% w/v) is prepared by dissolving 200 grams of reagent grade (BDH) hydroxylamine sulfate in 1 litre of distilled water. This solution is used to reduce the excess permanganate after digestion.

THIS SUBSTANCE IS CARCINOGENIC: AVOID CONTACT WITH THE SKIN.

- n) Stannous chloride solution; The solution (20% w/v) is prepared by dissolving 400 grams of reagent grade stannous chloride (BDH) in 2 litres of concentrated hydrochloric acid. This solution is used to reduce the mercury to its atomic form (Hg^0).
- o) Mercuric chloride stock solution; Prepared by dissolving 1.358 grams in one litre of distilled water containing 10 mls of concentrated nitric acid. Concentration 1,000 ug/ml.
- p) Methyl mercuric chloride stock solution; Prepared by dissolving 0.6258 grams of methyl mercuric chloride in 1000 mls of methanol (reagent grade). Concentration 500 ug/ml.
- q) Mercury standards; Working solutions containing 1 ug mercury per ml should be made up by making successive dilutions from the stock solution in distilled water. New working solutions should be made up weekly and the concentration of mercury in these should be within 1% of the old ones.

5. Procedure

Physical Preparation

The following is an outline of the treatment received by an individual fish. (27).

Initially, all adhering mucous is washed off and the fish is patted dry with paper towelling. It is measured (to 0.1 cm) and weighed (to 0.1 g) and the data is recorded.

Subsequent to weighing, the fish is placed on a wooden board covered with paper towelling. An incision is made with a stainless steel knife on the dorsal surface posterior to the nape, and the body is cut ventrally to a point below and posterior to the pectoral fin. The gut cavity is sliced open, and the liver removed, when analysis of this organ is required. It is transferred to an aluminum foil cup which is sealed and designated with a laboratory number. The sex of the fish is noted and recorded.

The fish is cut posteriorly along the dorsal surface. The epaxial musculature (muscle above the lateral line) is stripped from the skin and about 5 grams of it is placed into a clean stainless steel cup in which the tissue is ground for two minutes with a mechanical homogenizer (Virtis). Two to five grams of the homogenized tissue is then transferred with a stainless steel scoopula to an aluminum foil cup. The cup is sealed and designated with a laboratory number.

Samples are submitted to the analytical laboratory where they are frozen and stored until the analytical procedures are begun.

Analytical Procedure

- a) Thaw fish prior to weighing and homogenize again with a glass rod to ensure that the liquid-flesh partition, which occurs during freezing, does not contribute to imprecision or inaccuracy.
- b) About 0.3 to 0.5 g of homogenized fish tissue is injected into tared 30 ml micro-Kjeldahl flasks by means of an all-glass plunger device. The micro-Kjeldahl flasks are re-weighed on a single pan analytical balance to four significant figures, and the difference in weights due to the addition of the sample is recorded.
- c) The samples are prepared as part of a series of blanks, controls and samples called a "run".

A "run" of samples consists of

- 1) two reagent blanks,
- 2) two 1 ug inorganic standards,
- 3) two 1 ug organic standards,
- 4) two "control low" fish,
- 5) two "control high" fish,
- 6) one inorganic spike*,
- 7) one organic spike*,
- 8) twenty routine fish samples in duplicate.

"Control high" and "low" are fish that have been previously analyzed at least twenty times. "Control low" is in the 0.5 ug/ml range and "control high" is in the 3-4 ug/ml range.

*Spike samples are arbitrarily selected fish within the run that are spiked with known amounts of inorganic or organic standards (1 ug usually) to determine percent recovery.

- d) To the Kjeldahl flasks add 5.0 ml of digesting acid (4:1 v/v H_2SO_4 , HNO_3).
- e) Place flasks in oscillating water bath at 80°C and allow to digest for 3.5 to 4 hours.

NOTE: FOR FISH WITH HIGH FAT CONTENT (CARP, TUNA, STURGEON) OR IN DRIED FORM, A MINIMUM TIME OF FOUR HOURS IS REQUIRED. VISUAL INSPECTION OF THE DIGESTATE USUALLY PROVIDES A GOOD INDICATION OF COMPLETE DIGESTION (FLOATING FAT PARTICLES OR LIPIDS SEPARATING OUT INDICATE AN INCOMPLETE REACTION).

- f) When the digestates are clear, samples are removed from the water bath and saturated potassium permanganate solution is added dropwise to the Kjeldahl flasks, which are immersed in an ice bath to prevent over-heating. The samples are swirled in the cold bath and potassium permanganate is added until a persistent purple colour remains; an additional 5 ml are added and the flasks are placed back in the 80°C bath for 1.5 hours.

NOTE: AN INDICATION OF INCOMPLETE DIGESTION AT THIS POINT IS EXTREME FROTHING OF THE SAMPLE. IF THIS OCCURS THE SAMPLE SHOULD BE REWEIGHED AND REDIGESTED.

- g) The samples are removed from the bath and allowed to stand at room temperature overnight. If any of the samples become de-colourized during this period, more potassium permanganate must be added at the earliest opportunity.

Instrument Operation

FOR SET-UP OF FAAS SYSTEM (HILGER-WATTS ATOMSPEK)

IF ANOTHER INSTRUMENT IS USED CONSULT MANUFACTURER'S MANUAL.

- a) Install hollow cathode lamp in lamp housing and connect all cables.
- b) Set meter switch to proper lamp position number.
- c) Set lamp selector switch to position of selected lamp.
- d) Turn lamp resistors to minimum.
- e) Turn on power switch (main).
- f) Adjust lamp current to approximately 4.5 ma with lamp current resistor.

- g) Set mode switches to "Absorption" and "0-1".
- h) Set response to "Fast".
- i) Set E.H.T. resistor to "On" position.

Daily Warm-Up Procedure

- a) Timers should be set to allow unit to warm up for at least two hours prior to actual analysis.
- b) Warm up recorder (20 minutes).
- c) Follow procedure for cleaning apparatus.
- d) With all parts in place align cell on burner head to yield minimum absorption. Adjust lamp so that beam passes through centre of cell lenses. Adjust height of cell on burner if necessary.
- e) Set recommended wavelength for mercury (253.7 nm) with wavelength selector knob. Adjust to yield minimum absorbance in stepwise fashion. Finally, zero instrument with E.H.T. resistor.
- f) Place ice in coil bath and zero instrument after equilibrium is reached. Set slit width to about 11.5 microns.
- g) When absorbance is zeroed, set recorder to zero using the recorder zeroing control.
- h) Set recorder at "slow" (1 cm/minute) chart speed and 5.0 mV reading.
- i) Turn on tungsten element lamp to warm cell.
- j) Turn on air pump and set flow meter to 40. This will give a flow rate of about 5 litres per minute. The adjustment must be performed with a dreschel bottle of distilled water present in the system.
- k) Ensure that the drying agent (CaSO_4) is still hygroscopic.
- l) Check gas dispersion tubes (replace if worn).
- m) Check for leaks in connections.

6. Calculation and Reporting

There are three sets of standards analyzed.

- a) undigested inorganic standards,
- b) digested inorganic standards,
- c) digested organic standards.

The digested organic standards are chosen for calculations because they receive the same treatment as the samples and it has been shown that 80-100% of the mercury in fish is present in the organic form (28). The peak heights for the blanks and standards are measured and averaged, and standards are corrected for blank effect. The peak heights for the unknowns are then measured, blank corrected, and compared to the digested organic mercury standard, and a content of mercury in micrograms is recorded. The amount of mercury (μg) is then related to the fish weight taken $\mu g/g$ (concentration).

Formula:

$$\mu g/g \text{ mercury as Hg} = \frac{x - b}{(a - b) n}$$

x = peak height of sample

b = peak height of blank

a = average peak height of organic standard

n = weight in gram of fish aliquot

The samples are analyzed in duplicate and the percent deviation of the lowest of the two results from the mean must not exceed 10%. If it does, the sample is immediately reanalyzed in duplicate.

<u>Example:</u>	Duplicate results	0.89 $\mu g/g$ 0.85
	Mean results	0.87 $\mu g/g$
	% deviation	± 2.3

The percent deviation from the mean was less than $\pm 10\%$ so the result is acceptable.

Reporting

Range of mercury found, in $\mu g/g$ Reported mercury, in $\mu g/g$

<0.02	<0.02
0.02 to 0.09	1 significant figure.
0.10 to 0.99	2 significant figures.
1.0 to 99	2 significant figures.

THE DETERMINATION OF TOTAL MERCURY
IN BIOLOGICAL MATERIAL

Hot Block Heating, Mercury Meter Measurement

SUMMARY

Substance determined	Total Mercury
Interpretation of results	The total mercury concentration in biological material is the sum of all forms of mercury present in the sample. Total mercury in fish is generally interpreted as being indicative of the methyl mercury content, since methyl mercury is usually 80-100% of the total mercury in concentration.
Principle of Method	The biological material is digested in a mixture of sulfuric and nitric acids for approximately $1\frac{1}{2}$ hours or until white fumes are visible, then oxidized with potassium permanganate, and the excess permanganate is reduced with hydroxylamine sulfate and the divalent mercury reduced with stannous chloride. The vessel containing the sample is attached to the F.A.A.S. system and the sample aerated. The mercury now present as Hg^0 is purged through the system and measured spectrophotometrically.
Time required for analysis	Approximately 100 analyses in duplicate can be completed per day.
Range of Application	The absorption obeys Beers Law over the range 0.005 to 0.4 μg . Since 0.4 μg is approximately 80% full scale, dilution of samples containing more than 1 $\mu g/g$ is required.
Precision	$1.97 \pm 0.03 \mu g/g$ for ten determinations performed on a gelatin standard containing 2.00 $\mu g/g$ mercury.
Accuracy	From the precision data above, about 98%
Limit of Detection	About 0.005 $\mu g/g$ based on the normal weight of sample taken (0.5g). It is likely that greater sensitivity would be achieved by taking larger samples or by means of further electrical expansion on the readout equipment.
Interferences and short-comings	There are relatively few compounds that will interfere at the levels present in biological

SUMMARY

material. Of concern are chlorides and oxides of nitrogen, but a proper digestion procedure will eliminate these problems. Water vapour is eliminated by a water trap. Volatile aromatics and some diatomic gases can absorb at the mercury resonance wavelength, but these are normally absent after the digestion step.

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of sample

A minimum *5 grams* of homogenized material is desirable.

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and Sample
container

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considerations

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IN BIOLOGICAL MATERIAL

Hot Block Heating, Mercury Meter Measurement

1. Introduction

Frozen samples are thawed and homogenized again with a glass stirring rod to redistribute any liquid that could partition out during thawing. Approximately 0.3 to 0.5 g of homogenized tissue are accurately weighed into 50 ml pyrex culture tubes. Five ml of a 4:1 volumetric ratio of sulfuric-nitric acids are added. The tubes are then placed in an aluminum block with holes capable of holding 50 ml culture tubes. The block is heated to 260°C by means of a hot plate and the samples allowed to fume (white SO₃). The samples are then allowed to cool and saturated potassium permanganate solution is added as a further oxidant. The samples are allowed to stand for ½ hour and hydroxylamine sulfate added to reduce any excess permanganate or manganese dioxide. Immediately prior to purging the sample with air, stannous chloride is added to reduce the mercury to its atomic state. The mercury vapour is then passed through the FAAS system and the resultant absorption is measured spectrophotometrically and presented on a recorder.

2. Interferences and shortcomings

There are a number of elements or their oxides that can interfere both chemically and/or spectrally with mercury when analyzed using FAAS. Practically none of these are present in biological material at sufficient levels to cause problems.

Selenium, tellurium, antimony, bismuth and arsenic are reported to interfere chemically, but this effect has not been observed in biological samples in this laboratory.

A group of interfering substances that cause the problems because of their positive spectral interference are water vapour, NO₂, Cl₂ and any volatile organic solvents that absorb at 253.7 nm.

Water vapour is eliminated from the system by the insertion of a coil in an ice bath followed immediately by a water trap prior to the optical cell.

NO₂, CO₂ and Cl₂ should not be present after the digestion step normally performed.

Organic solvents (acetone, chloroform, etc.) should not be used in the laboratory or for cleaning glassware.

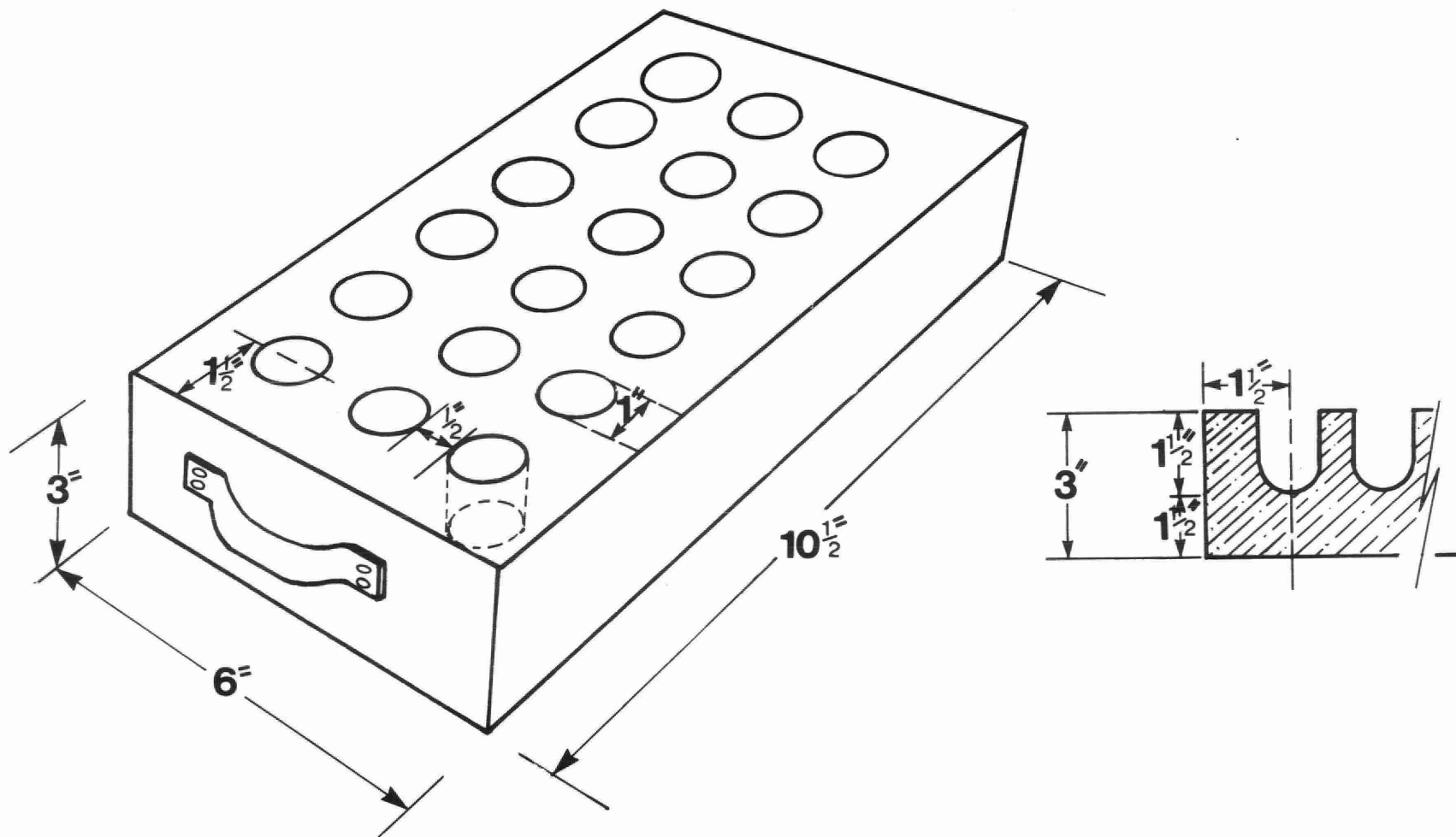


Diagram 2

Aluminum Hotblock

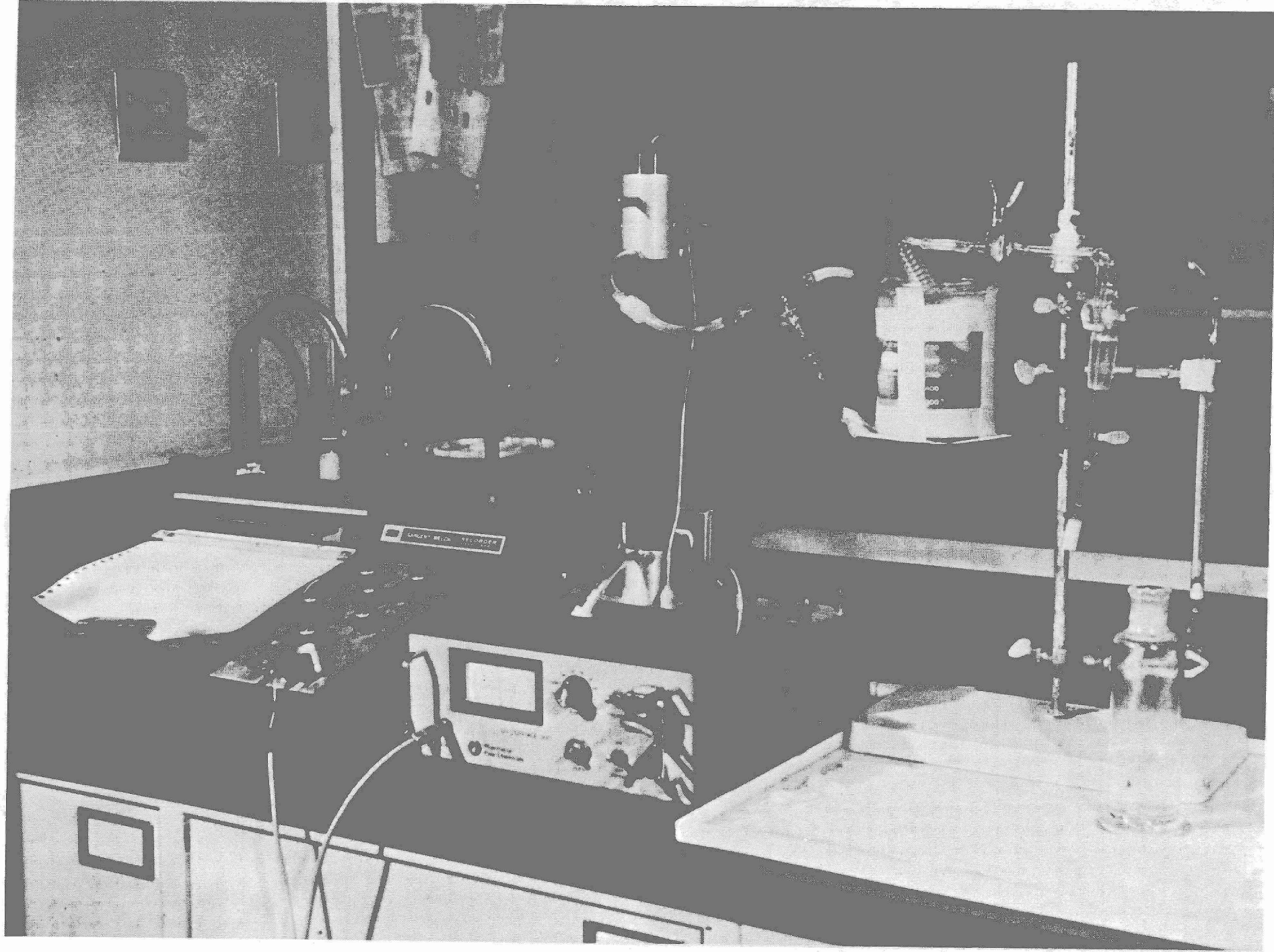


Plate 2

Mercury meter & aeration apparatus

3. Apparatus

REFER TO DIAGRAMS 1 AND 2 AND PLATES 2 AND 3 FOR A
PICTORIAL VIEW OF THE APPARATUS.

- a) Laboratory Data Control U.V. Monitor.
- b) Sargent Welch recorder; (model SRG).
- c) Aluminum block (diagram 2).
- d) Culture tubes, 50 ml.
- e) Analytical Balance; single pan (Mettler).
- f) Dreschel bottle; 250 ml volume, Quickfit (BS2461)
with a 24/29 ground joint.
- g) Plunger device; composed of two parts (1). Pyrex
tubing, I.D. 9 mm; O.D. 11 mm, and 20 cm in length.
(2). Glass rod 8 mm in diameter, 25 cm in length.
- h) Mixing coil; as supplied for Technicon Auto-analyzer,
(13 cm long).
- i) Flowmeter; Gilmont (size #3).
- j) Hot plate, capable of maintaining 300°C.
- k) Gas dispersion tube; with fritted glass cylinder,
250 mm length, stem diameter 8 mm.
- l) PTFE stopcock; three way, Quickfit 24/29 used for
directing flow of air (1). bypassing sample and
(2) through sample, thus aerating it.
- m) Gas bubble counter; (Canlab #C7778-C) used as water
droplet trap.
- n) Compressed air.

ALL GLASSWARE AND APPARATUS MUST BE THOROUGHLY
CLEANED PRIOR TO USE.

Cleaning of Apparatus

- a) The optical cell should be cleaned once a week and/or
following contamination with excessively high level
mercury samples. Rinse cell with (1) water
(2) nitric acid
(3) methanol

THE CELL SHOULD THEN BE BLOWN DRY WITH AIR.

- b) The teflon stopcock should be cleaned every week (minimum) with chromic acid, water, methanol and then air-dried.
- c) All polyethylene and glass tubing should either be replaced or rinsed with water, followed by methanol and blown dry with a stream of air.
- d) The dreschel bottle should be cleaned thoroughly every day or after an extremely high sample, with a cleaning solution containing 1% EDTA in 20% NaOH. Bottles are usually soaked in this solution for twelve hours.

ONLY ONE BOTTLE IS USED FOR STANDARDS AND SAMPLES DURING A RUN BECAUSE THERE IS A "BOTTLE EFFECT" WHICH CAUSES SLIGHT BUT SIGNIFICANT DIFFERENCES IN READINGS FROM BOTTLE TO BOTTLE.

- e) Culture tubes are cleaned immediately after use with
 - (1) soap and water
 - (2) chromic acid
 - (3) distilled water

TUBES MUST BE DRY BEFORE RE-USE.

4. Reagents

ALL REAGENTS USED SHOULD BE ANALYZED IN THE LABORATORY AND ONLY THOSE WITH A SUFFICIENTLY LOW MERCURY CONTENT SHOULD BE RETAINED.

- a) Sulfuric acid; reagent grade, concentrated. Experience with a variety of acids indicate that either Baker Analyzed or McArthur Chemicals sulfuric acids are acceptable. Reagents other than those mentioned in this section may possess the required degree of purity.
- b) Nitric acid; reagent grade, concentrated. Of several brands investigated, only Baker Analyzed reagent has been found satisfactory.
- c) Hydrochloric acid; reagent grade, concentrated. Mallinckrodt reagent has been found satisfactory.
- d) Potassium permanganate; analytical reagent. BDH Analar crystals have been found satisfactory.
- e) Hydroxylamine sulfate; laboratory reagent, BDH reagent is satisfactory.
- f) Stannous chloride; analytical reagent. BDH Analar reagent is satisfactory.
- g) Sodium Hydroxide; Certified A.C.S. Electrolytic Pellets.
- h) Ethylenediaminetetra-acetic acid; laboratory reagent. BDH Disodium salt is satisfactory.
- i) Mercuric chloride; reagent grade. As supplied by Mallinckrodt Chemicals.
- j) Methyl Mercuric Chloride; reagent grade. Alfa Organics is a satisfactory supplier.
- k) Digestion Acid

The mixture contains a volumetric ratio of *four* parts sulfuric acid (concentrated) to *one* part nitric acid (concentrated).

MIXING CAUSES AN EXOTHERMIC REACTION; ACCORDINGLY EXTREME CARE SHOULD BE EXERCISED DURING THIS STEP.

l) Potassium Permanganate solution

The solution should be saturated (60 g/l) at room temperature, then allowed to stand for at least 48 hours in sunlight before use, to form small amounts of MnO_2 , which slowly settles out. This step greatly reduces the risk of mercury contamination from the $KMnO_4$.

m) Hydroxylamine Sulfate solution

The solution (20% w/v) is prepared by dissolving 200 grams of reagent grade (BDH) hydroxylamine sulfate in 1 litre of distilled water. This solution is used to reduce the excess permanganate after digestion.

n) Stannous chloride solution

The solution (20% w/v) is prepared by dissolving 400 grams of reagent grade stannous chloride (BDH) in 2 litres of concentrated hydrochloric acid. This solution is used to reduce the mercury to its atomic form (Hg^0).

o) Mercuric chloride stock solution

Prepared by dissolving 1.358 grams in one litre of distilled water containing 10 mls of concentrated nitric acid. Concentration 1,000 ug/ml.

p) Methyl mercuric chloride stock solution

Prepared by dissolving 0.6258 grams of methyl mercuric chloride in 1000 mls of methanol (reagent grade). Concentration 500 ug/ml.

q) Mercury standards

Working solutions containing 1 μg mercury per ml should be made up by making successive dilutions from the stock solution in distilled water. New working solutions should be made up weekly and the concentration of mercury in these should be within 1% of the old ones.

5. Procedure

Physical Preparation

The following is an outline of the treatment received by an individual fish (27).

Initially, all adhering mucous is washed off and the fish is patted dry with paper towelling. It is measured (to 0.1 cm) and weighed (to 0.1g) and the data is recorded.

Subsequent to weighing, the fish is placed on a wooden board covered with paper towelling. An incision is made with a stainless steel knife on the dorsal surface posterior to the nape, and the body is cut ventrally to a point below and posterior to the pectoral fin. The gut cavity is sliced open, and the liver removed, when analysis of this organ is required. It is transferred to an aluminum foil cup which is sealed and designated with a laboratory number. The sex of the fish is noted and recorded.

The fish is cut posteriorly along the dorsal surface. The epaxial musculature (muscle above the lateral line) is stripped from the skin and about 5 grams of it is placed into a clean stainless steel cup in which the tissue is ground for *two minutes* with a mechanical homogenizer (Virtis). *Two to five grams* of the homogenized tissue is then transferred with a stainless steel scoopula to an aluminum foil cup. The cup is sealed and designated with a laboratory number.

Samples are submitted to the analytical laboratory where they are frozen and stored until the analytical procedures are begun.

Analytical Procedure

- a) Thaw fish prior to weighing and homogenize again with a glass rod to ensure that the liquid-flesh partition, which occurs during freezing, does not contribute to imprecision or inaccuracy.
- b) About 0.3 to 0.5 g of homogenized fish tissue is injected into tared 50 ml pyrex culture tubes by means of an all-glass plunger device. The culture tubes are re-weighed on a single pan analytical balance to *four* significant figures, and the difference in weights due to the addition of the sample is recorded.
- c) The samples are prepared as part of a series of blanks, controls and samples called a "run".
A "run" of samples consists of -
 - 1) *two* reagent blanks,
 - 2) An organic standard curve consisting of 0.1, 0.2, 0.3 and 0.4 µg in duplicate,
 - 3) *two* "control low" fish,
 - 4) *two* "control high" fish,
 - 5) *one* inorganic spike*,
 - 6) *one* organic spike*,
 - 7) *twenty* routine fish samples in duplicate.

"Control high" and "low" are fish that have been previously analyzed at least *twenty* times. "Control

low" is in the $0.5 \mu\text{g/g}$ range and "Control high" is in the $2-3 \mu\text{g/g}$ range.

*Spike samples are arbitrarily selected fish within the run that are spiked with known amounts of inorganic or organic standards ($0.02 \mu\text{g}$ usually) to determine percent recovery.

- d) To the culture tubes add 5.0 ml of digesting acid ($4:1 \text{ v/v } \text{H}_2\text{SO}_4, \text{HNO}_3$).
- e) Place tubes in an aluminum block and heat at 260°C for approximately $1\frac{1}{2}$ hours or until continuous white fuming takes place. After cooling the digestate should be completely clear.
- f) The samples are then swirled in an ice bath while enough potassium permanganate solution is added to maintain a purple colour (less than 2 ml).
- g) The samples are allowed to stand for $\frac{1}{2}$ hour before reduction.
- h) Reduce excess permanganate by adding 1 ml of 20% hydroxylamine sulfate solution. Before addition of the hydroxylamine sulfate solution approximately 20 ml of distilled water is added to prevent generation of excessive heat and the remote possibility of sample foaming.
- i) Decant the sample into a dreschel bottle and rinse the Kjeldahl flask thoroughly with distilled water. The final volume in the dreschel bottle should be 50 ml .
- j) Add 3.0 ml of 20% stannous chloride solution to reduce the mercury.
$$(\text{Hg}^{+2} + \text{Sn}^{+2} \rightarrow \text{Hg}^0 + \text{Sn}^{+4})$$
- k) Immediately attach the dreschel bottle to the apparatus and aerate the sample.
- l) After maximum absorbance has been attained, flush the system by aerating a bottle containing distilled water only, until the original baseline is reached on the recorder.

Special Precautions

- i) Periodically the instrument should be checked for drift from zero position.

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- g) The samples are allowed to stand for $\frac{1}{2}$ hour before reduction.
- h) Reduce excess permanganate by adding 1 ml of 20% hydroxylamine sulfate solution. Before addition of the hydroxylamine sulfate solution approximately 20 ml of distilled water is added to prevent generation of excessive heat and the remote possibility of sample foaming.
- i) Decant the sample into a dreschel bottle and rinse the Kjeldahl flask thoroughly with distilled water. The final volume in the dreschel bottle should be 50 ml .
- j) Add 3.0 ml of 20% stannous chloride solution to reduce the mercury.



- k) Immediately attach the dreschel bottle to the apparatus and aerate the sample.
- l) After maximum absorbance has been attained, flush the system by aerating a bottle containing distilled water only, until the original baseline is reached on the recorder.

Special Precautions

- i) Periodically the instrument should be checked for drift from zero position.

- ii) If any changes to the system are made during the run, new standards should be analyzed to determine variations in sensitivity.
- iii) Moisture content in the water trap should be periodically checked. Empty as needed to prevent bubbling.

Instrument Operation

*FOR SET-UP OF FAAS SYSTEM (L.D.C. MERCURY METER).
IF ANOTHER INSTRUMENT IS USED CONSULT MANUFACTURERS
MANUAL.*

- a) If equipment is installed as instructed in the manual, no problems should arise.
- b) Meter and recorder should be allowed to warm-up for $\frac{1}{2}$ hour prior to use.
- c) Follow procedure for cleaning apparatus.
- d) Meter reading should not be allowed to exceed 0.4.

*A READING ABOVE THIS LEVEL INDICATES PRESENCE OF
MOISTURE IN THE CELL.*

- e) When the system is equilibrated, set recorder to zero using the controls on the U.V. detector.
- f) Set recorder at "slow" (1 cm/minute) chart speed and 10 mv reading. The UV monitor range setting should be 0.64.
- g) Turn on compressed air supply and set flow to 30 (3.4 l/min.) The adjustment must be performed with a dreschel bottle of distilled water present in the system.
- h) Check gas dispersion tubes (replace if worn).
- i) Check for leaks in connections.
- j) Run at least a standard curve in duplicate (0.1, 0.2, 0.3, 0.4 Hg units). If reproducibility is poor the set-up procedure should be repeated; the pipetting technique re-examined, and the possibility of a leak in the system checked.
- k) Check water trap periodically for water content. Bubbling and the attendant possibility of water entering the cell must be avoided.

6. Calculation and Reporting

There are three sets of standards analyzed.

- a) undigested inorganic standards,
- b) digested inorganic standards,
- c) digested organic standards.

The digested organic standards are chosen for calculations because they receive the same treatment as the samples and it has been shown that 80-100% of the mercury in fish is present in the organic form. The peak heights for the blanks and standards are measured and averaged. Standards are corrected for blank effect and a standard curve is constructed. The peak heights for the unknowns are then measured, blank corrected, and compared to the digested organic mercury standard curve, and the mercury content in micrograms is recorded. The amount of mercury (μg) is then related to the fish weight taken $\mu\text{g/g}$ (concentration).

Formula:

$$\mu\text{g/g mercury as Hg} = \frac{x - b}{(a - b) n}$$

x = peak height of sample

b = peak height of blank

a = average peak height of organic standard

n = weight in gram of fish aliquot

The samples are analyzed in duplicate and the percent deviation of the lowest of the two results from the mean must not exceed 10%. If it does, the sample is immediately reanalyzed in duplicate.

Example:

Duplicate results	0.89 $\mu\text{g/g}$
	0.85
Mean results	0.87 $\mu\text{g/g}$
% deviation	± 2.3

The percent deviation from the mean was less than $\pm 10\%$ so the result is acceptable.

Reporting

Range of mercury found, in $\mu\text{g/g}$	Reported mercury, in $\mu\text{g/g}$
<0.02	<0.02
0.02 to 0.09	1 significant figure.
0.10 to 0.99	2 significant figures.
1.0 to 99	2 significant figures.

THE DETERMINATION OF TOTAL MERCURY
BY COMBUSTION-AMALGAMATION

SUMMARY

Substance determined.	Total Mercury.
Interpretation of Results.	Results are reported in $\mu\text{g/l}$ or ng/l based on the "wet" weight of the sample.
Principle of Method.	Mercury is released from the sample into a stream of oxygen by combustion at approximately 800°C in a high-frequency induction furnace. The mercury vapor and combustion products are passed through a sodium hydroxide wash, a constant water vapor trap and a calcium sulfate water trap, in order to remove interferences. The mercury is then collected on a gold trap (gold wire) and the amalgam is reheated (450°) to release the mercury, thus avoiding the effects of most possible interferences. The mercury is then quantitated by use of a mercury meter.
Time required for Analysis.	A single analysis requires about <i>ten minutes</i> . About <i>forty</i> determinations can be completed in one day.
Range of application.	With a sample weight of 0.2g , a range of $0.01 - 5 \mu\text{g/g}$.
Precision and accuracy.	An average recovery of $96.6 \pm 5\%$ was attained on four samples (analyzed by sixteen reputable laboratories) containing 0.77 to $4.6 \mu\text{g/g}$.
Limit of detection.	10 ng/g at present (January 1973).
Interferences and shortcomings.	The extreme sensitivity of this method causes problems when samples containing greater than $5 \mu\text{g/g}$ of mercury are analyzed because smaller aliquots must be taken, resulting in increasingly large errors due to sample heterogeneity. Samples containing large amounts of sulfide (such as hair samples) can cause problems because the sodium hydroxide trap becomes saturated with SO_2 , causing an unstable baseline.
Minimum volume of sample.	0.5 gram of biological or sediment sample, 10 ml of aqueous sample.
Preservation and sample container.	Biological samples should be quick-frozen and placed in a clean mercury-free container.

SUMMARY

Preservation and sample container (cont'd)	Sediment samples should be stored in a clean, mercury-free container. Aqueous samples should be acidified with nitric acid and stored in a clean, mercury-free container.
Safety considerations	The high voltage and radio frequency radiation basic to the system described herein require that special precautions be observed.

THE DETERMINATION OF TOTAL MERCURY
BY COMBUSTION-AMALGAMATION

1. Introduction

Aliquots of not more than 0.2 g of fish tissue, sediment or 0.2 ml of aqueous solution are placed in a ceramic crucible and heated in an oxygen enriched atmosphere for 3.5 minutes in one coil of a twin coil radio-frequency induction furnace. The released mercury vapor and combustion products are carried by the oxygen stream (30) through a sodium hydroxide scrubber, a water condenser and finally through a calcium sulfate moisture trap (29).

The mercury, now in its atomic stage (Hg^0), is collected on gold wire (five grams of 24 gauge wire cut into approximately 3 mm lengths). The mercury readily amalgamates with the gold (31, 32). The gold-mercury amalgam is then heated by a second coil at approximately 450°C until all the mercury is volatilized, passed through an absorption cell, and measured by an L.D.C. mercury monitor.

2. Interferences and shortcomings

The method is practically interference free, as most volatile organics, sulfides and water vapor are removed by the NaOH scrubber and water traps.

Any compounds capable of absorbing radiation at 253.7 nm that escape the previously mentioned precautions will generally pass the gold trap unobstructed, and will cause absorption in the mercury measuring system before any mercury is released from the gold trap. The gold-mercury amalgam in the second coil is not heated until any peak shift from interferences has stabilized. It is noteworthy that very few samples show significant observable interferences at this point.

3. Apparatus

- a) Leco Induction Furnace; Model 523-300, twin tube furnace equipped with type "L" conversion for combustion sulfur analyses of hydrocarbons.
- b) Variable transformer; Leco #84, for control of power input to the induction furnace.
- c) Ceramic crucibles; Leco #528035, for containing samples.
- d) Vycor insert crucibles; Leco #550183 for use with aqueous samples and standards.

- e) Silicon carbide crucible covers; Leco #763212 (20 x 3 mm) used to couple with induction coil thus heating sample by reflectance.
- f) Spectrophotometer; Laboratory Data Control Model 1235 mercury monitor, used as an extremely sensitive F.A.A.S. system.
- g) Recorder, Sargent Welch (model SR6), equipped with a disc integrator.
- h) Gas washing bottle; with a 29/40 joint 175 ml volume, containing 35 ml of 6% (w/v) sodium hydroxide in distilled water. Renew solution daily. The solution should be prepared prior to use, and aerated to remove traces of mercury from sodium hydroxide reagent.
- i) Water condenser; separable plastic vacuum trap (30 x 160 mm) with body of tube immersed in ice bath. Empty water as needed to prevent bubbling.
- j) Heating tape; 2.5 x 610 cm "Electrothermal", with Variac to control heat (Power slot 120 v). Tape is wrapped around Teflon tubing to prevent acidic residue condensation within.
- k) Teflon tubing; 8 mm I.D. heat and acid resistant.
- l) Drying tubing; Plastic 175 x 20 mm, containing CaSO_4 . Replace CaSO_4 daily.
- m) Amalgamator; Vycor glass, containing glass wool and gold wire (see drawing).
- n) Flowmeter; Gilmont (size #3).
- o) Syringes, Eppendorf, 50, 25, 10, 5 μl .

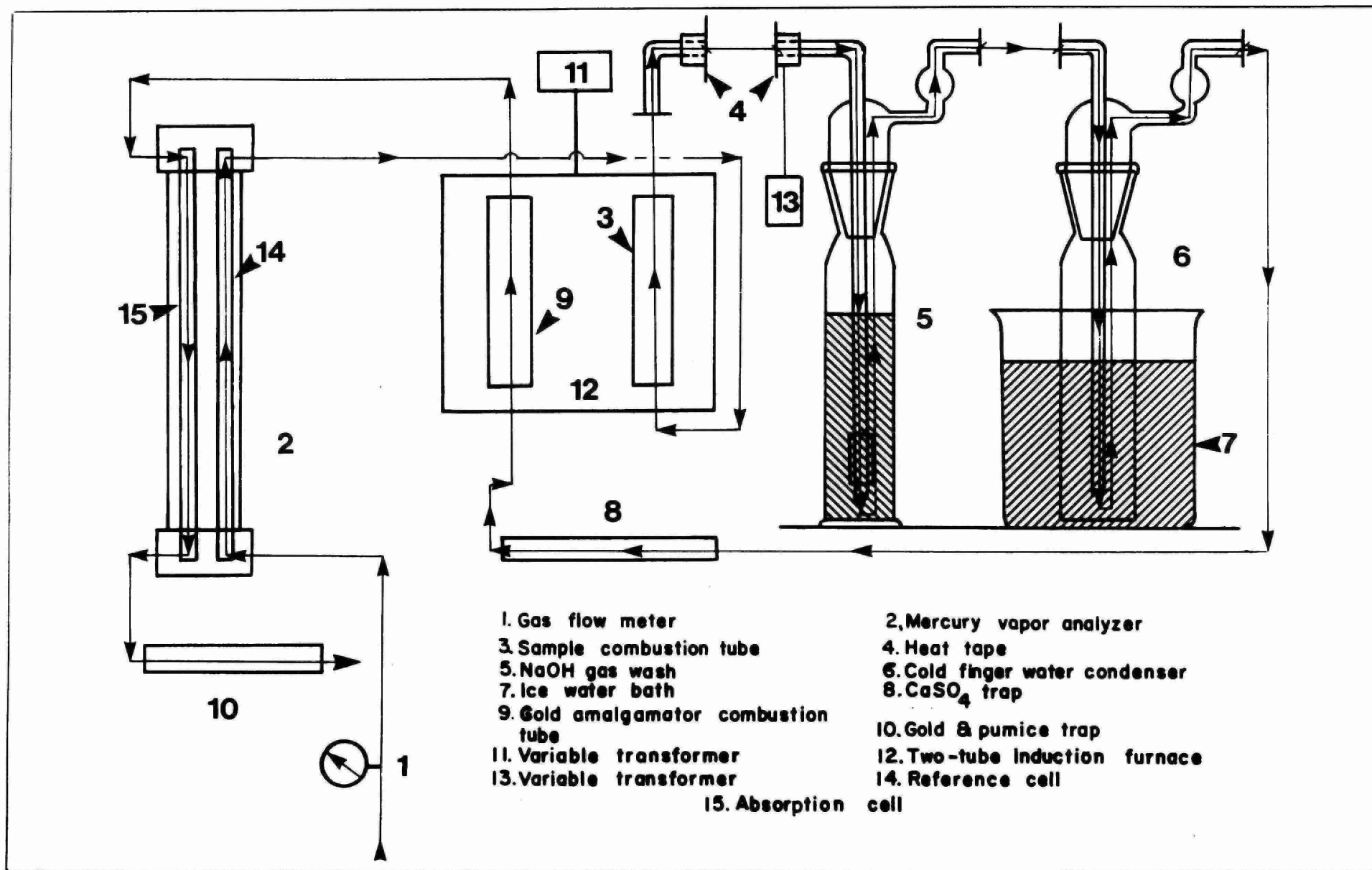


Diagram 3

Mercury Meter System

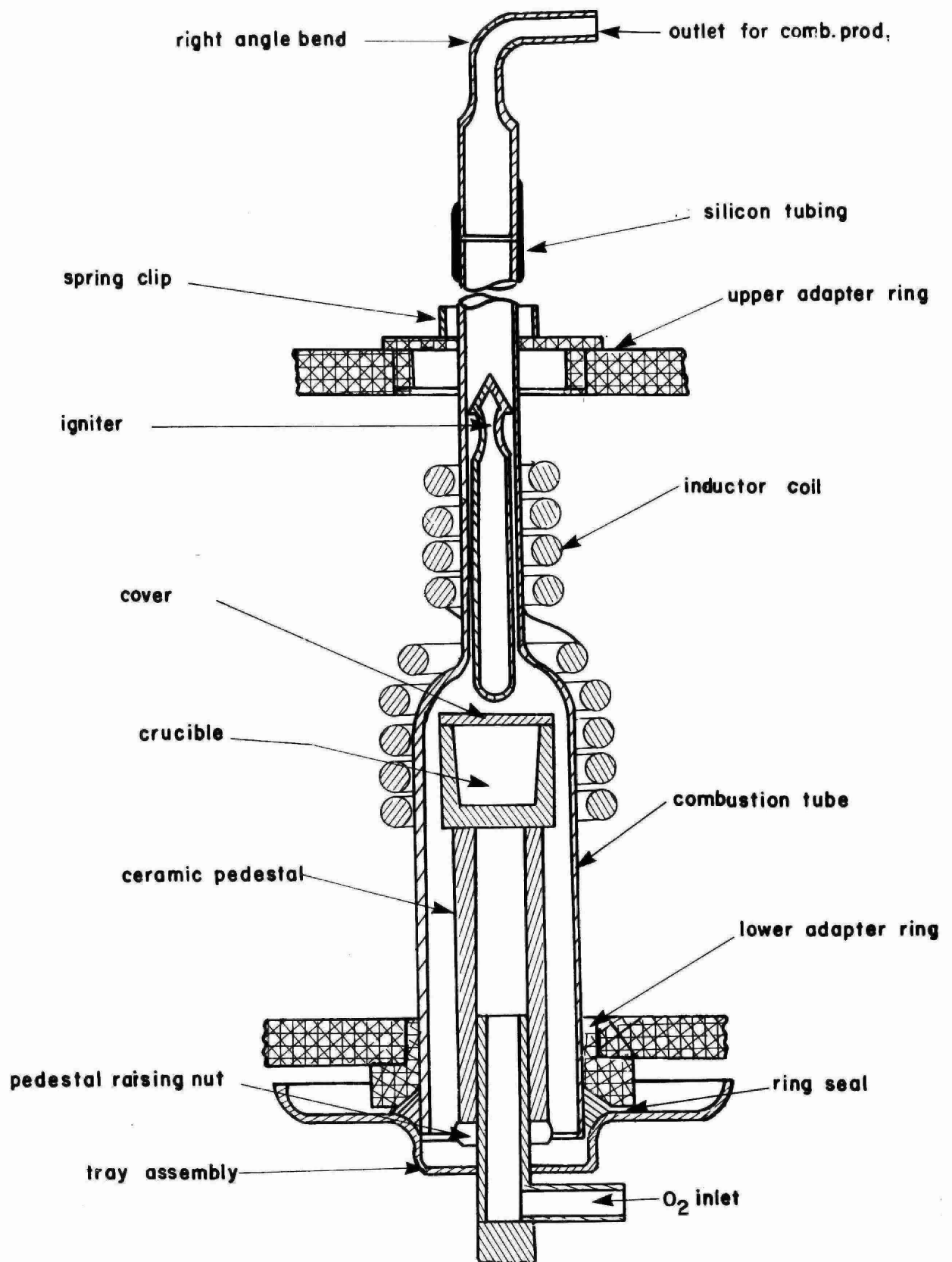


Diagram 4

Inductor Coil Detail

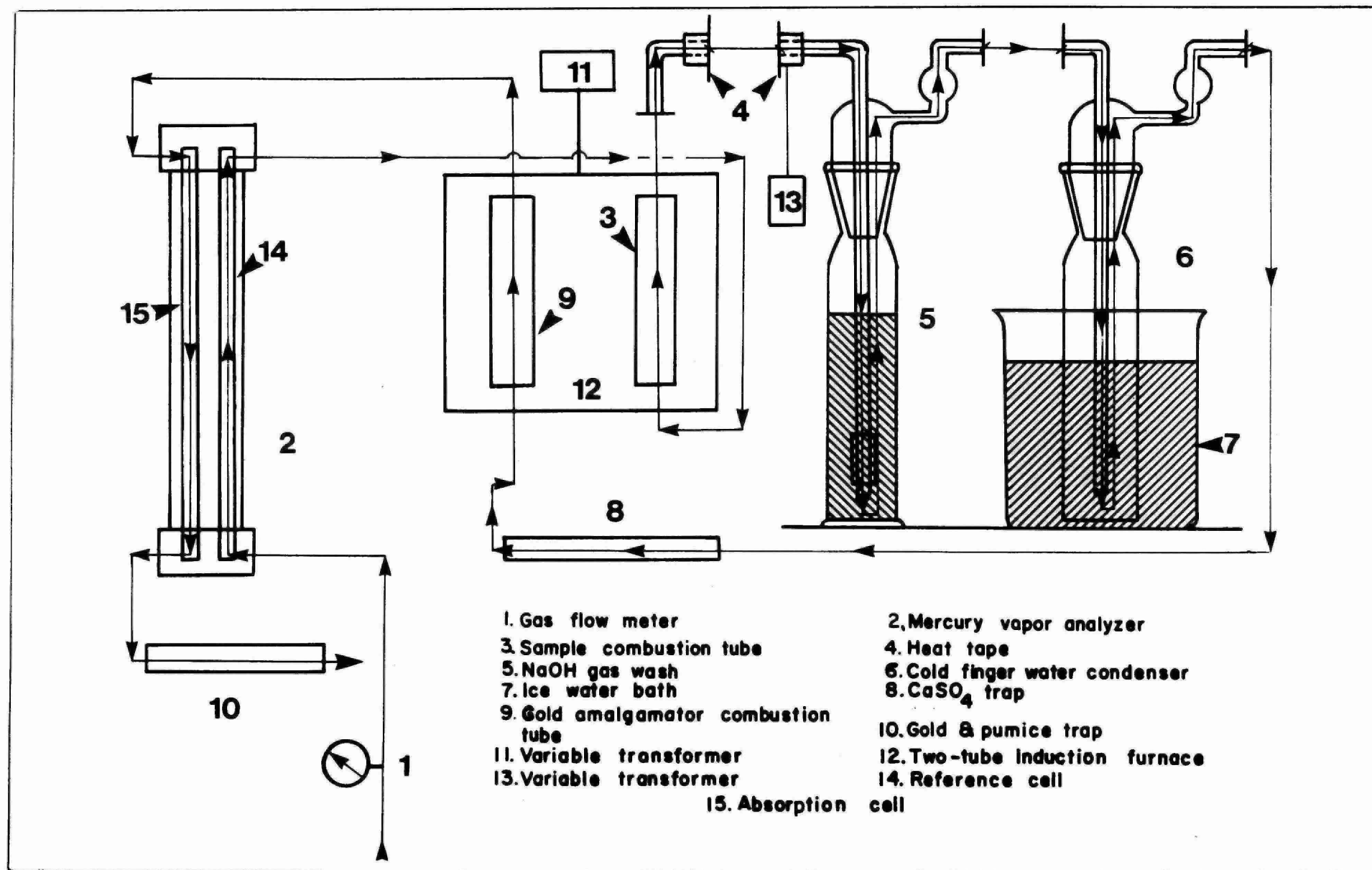


Diagram 3

Mercury Meter System

- e) Pipet an appropriate standard (usually 0.1 ml of 1 µg/ml at the 0.32 setting on L.D.C. readout) into a Vycor insert crucible, place insert into a ceramic crucible, and cover with the porous silicon carbide cover.

THE USE OF THE VYCOR INSERT CRUCIBLE IS REQUIRED FOR AQUEOUS SOLUTIONS BECAUSE THE CERAMIC CRUCIBLES ARE TOO POROUS TO BE USED DIRECTLY.

- f) Place the covered ceramic crucible on the pedestal of the right side inductor coil, lift the raising mechanism and lock it into the operational position. At this point the high voltage (red) light should be illuminated and there should be an increase in the grid and plate amperage readings.
- g) Heat the crucible for 3.5 minutes. The left side inductor coil lifting mechanism holding the gold trap must remain in the lowered position during this part of the procedure.
- h) After combustion of the sample, release the heating mechanism but do not stop the flow of oxygen.
- i) Lift the left side raising mechanism supporting the gold trap. This activates the left coil, causing it to heat the gold trap for approximately 1.5 minutes or until the recorder pen returns to baseline.
- j) The gold trap should be allowed to cool for at least two minutes before re-use; failure to do so will cause premature release of mercury on the following sample.
- k) The samples can be weighed into the ceramic crucibles and treated the same as the standards. Standards are chosen to match the expected level of mercury in the samples being analyzed. A range of standards are run to provide a calibration curve. Precision is checked by running at least five identical standards. Relative standard deviation must be less than 10%.
- l) Mercury concentrations are calculated based on peak areas and an integrating recorder greatly simplifies the work involved in the calculation.

THE PEAK HEIGHTS ARE NOT TO BE USED BECAUSE OF SMALL CHANGES IN THE HEATING AND MERCURY-RELEASING CHARACTERISTICS OF THE GOLD AMALGAM, WHICH RESULT IN IMPRECISION.

4. Reagents

- a) Sodium hydroxide solution; 6% w/v.

An ultra-pure reagent should be used and aerated prior to use.

- b) Mercury Chloride Stock Solution

Prepared by dissolving 1.358 grams in one litre of distilled water containing 10 mls of concentrated nitric acid (1000 ug Hg/ml).

- c) Methyl Mercury Chloride Stock Solution

Prepared by dissolving 0.6258 grams of methyl mercuric chloride in 1000 mls of methanol (Reagent grade). (500 ug Hg/ml).

- d) Mercury Standards

Working solutions containing 1 ug mercury per ml should be prepared by making successive dilutions from the stock solution in distilled water. New working solutions should be made up weekly and should be checked for mercury content against the "old" working solutions. Agreement should be within 1%.

- e) Oxygen supply

As supplied by Gas Dynamics Limited.

5. Procedure

- a) The furnace should be set up as described in the Leco procedure for the determination of sulfur in hydrocarbons. The equipment set up is shown in Plate 3, and a schematic is provided in Diagram 3. The right side inductor coil should be set up as shown in Diagram 4, and the left side as shown in Diagram 5.
- b) Set oxygen flow through the apparatus at 1 litre per minute and check system for leaks.
- c) Adjust setting on the furnace variac to 80 and set grid tap to low. The heating tape should be generating enough heat to prevent condensation of moisture on the inside of the Teflon tubing that connects the combustion tube with the caustic trap.
- d) Zero the mercury monitor and recorder.

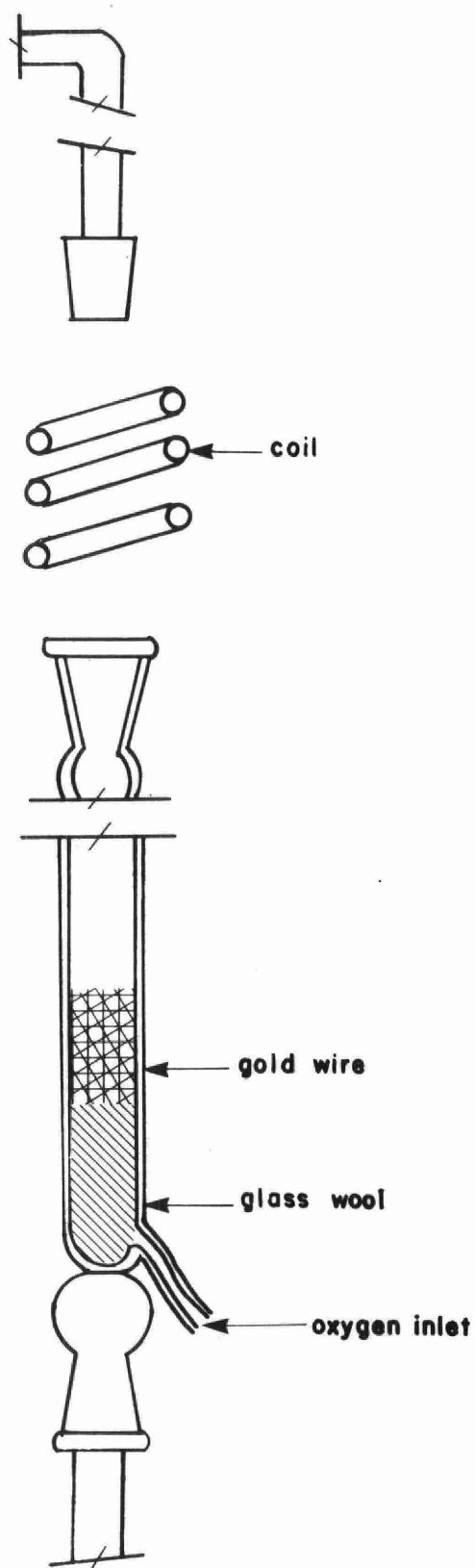


Diagram 5

Gold Trap-Inductor Coil

4. Reagents

- a) Sodium hydroxide solution; 6% w/v.

An ultra-pure reagent should be used and aerated prior to use.

- b) Mercury Chloride Stock Solution

Prepared by dissolving 1.358 grams in one litre of distilled water containing 10 mls of concentrated nitric acid (1000 ug Hg/ml).

- c) Methyl Mercury Chloride Stock Solution

Prepared by dissolving 0.6258 grams of methyl mercuric chloride in 1000 mls of methanol (Reagent grade). (500 ug Hg/ml).

- d) Mercury Standards

Working solutions containing 1 ug mercury per ml should be prepared by making successive dilutions from the stock solution in distilled water. New working solutions should be made up weekly and should be checked for mercury content against the "old" working solutions. Agreement should be within 1%.

- e) Oxygen supply

As supplied by Gas Dynamics Limited.

5. Procedure

- a) The furnace should be set up as described in the Leco procedure for the determination of sulfur in hydrocarbons. The equipment set up is shown in Plate 3, and a schematic is provided in Diagram 3. The right side inductor coil should be set up as shown in Diagram 4, and the left side as shown in Diagram 5.
- b) Set oxygen flow through the apparatus at 1 litre per minute and check system for leaks.
- c) Adjust setting on the furnace variac to 80 and set grid tap to low. The heating tape should be generating enough heat to prevent condensation of moisture on the inside of the Teflon tubing that connects the combustion tube with the caustic trap.
- d) Zero the mercury monitor and recorder.

- e) Pipet an appropriate standard (usually 0.1 ml of 1 µg/ml at the 0.32 setting on L.D.C. readout) into a Vycor insert crucible, place insert into a ceramic crucible, and cover with the porous silicon carbide cover.

THE USE OF THE VYCOR INSERT CRUCIBLE IS REQUIRED FOR AQUEOUS SOLUTIONS BECAUSE THE CERAMIC CRUCIBLES ARE TOO POROUS TO BE USED DIRECTLY.

- f) Place the covered ceramic crucible on the pedestal of the right side inductor coil, lift the raising mechanism and lock it into the operational position. At this point the high voltage (red) light should be illuminated and there should be an increase in the grid and plate amperage readings.
- g) Heat the crucible for 3.5 minutes. The left side inductor coil lifting mechanism holding the gold trap must remain in the lowered position during this part of the procedure.
- h) After combustion of the sample, release the heating mechanism but do not stop the flow of oxygen.
- i) Lift the left side raising mechanism supporting the gold trap. This activates the left coil, causing it to heat the gold trap for approximately 1.5 minutes or until the recorder pen returns to baseline.
- j) The gold trap should be allowed to cool for at least two minutes before re-use; failure to do so will cause premature release of mercury on the following sample.
- k) The samples can be weighed into the ceramic crucibles and treated the same as the standards. Standards are chosen to match the expected level of mercury in the samples being analyzed. A range of standards are run to provide a calibration curve. Precision is checked by running at least five identical standards. Relative standard deviation must be less than 10%.
- l) Mercury concentrations are calculated based on peak areas and an integrating recorder greatly simplifies the work involved in the calculation.

THE PEAK HEIGHTS ARE NOT TO BE USED BECAUSE OF SMALL CHANGES IN THE HEATING AND MERCURY-RELEASING CHARACTERISTICS OF THE GOLD AMALGAM, WHICH RESULT IN IMPRECISION.

- m) The area under the curve for a sample is compared to the standard calibration curve and the concentration of mercury is extrapolated using this data.

6. Calculation and Reporting

Formula -

$$\frac{(a - c) \times d}{(b - c) \times e} = \text{ng/g Mercury as Hg}$$

a = area under curve of sample

b = area under curve of standard

c = area under curve of blank

d = standard in ng

e = weight of sample in grams

Reporting

Range in ng/g	Reported in ng/g
50 ng/g	50 ng/g
50.0 to 100	3 significant figures
100 to 1000	3 significant figures

THE DETERMINATION OF TOTAL MERCURY
IN SEDIMENT

SUMMARY

Substance determined.	Total mercury in sediment.						
Interpretation of results.	All sediment data is reported on a dry weight basis, drying factors are calculated on separate aliquots but the mercury determination is always performed on the wet, homogenized sediment.						
Principle of method.	A weighed portion of sample is digested in dilute aqua regia. Addition of potassium permanganate makes this acidic medium strongly oxidizing and the samples are reheated. The samples are filtered and reduced with hydroxylamine sulfate and stannous chloride solutions. The sample is attached to the F.A.A.S. system and aerated. The reduced mercury is purged through the A.A.S. system, causing absorption of the mercury resonance wavelength. This is measured spectrophotometrically and is presented as a peak on a recorder. The sample peak height is then compared to digested standard peak heights and the mercury content of the sample is determined from this ratio.						
Time required for analysis.	A single analysis requires approximately <i>one day</i> . However, a set of <i>twelve</i> samples in duplicate, with blanks, standards, and spikes can be completed in <i>one day</i> when the samples are tested in batches.						
Range of application.	Without extension of detection limits by dilution or recorder adjustments, the range is 0.02 to 1.75 µg/g.						
Precision and accuracy.	±2% at the 50 µg/g level. The Environmental Protection Agency in the U.S. supplied two round-robin sediments to the M.O.E. and the following data compares the mean results of forty-eight participating laboratories to those obtained using the procedure described herein. (33).						
	<table><tr><th>Mean µg/g</th><th>M.O.E. (Aqua Regia Digestion)</th></tr><tr><td>109.5</td><td>105 (96% recovery)</td></tr><tr><td>43.5</td><td>44.2 (102% recovery)</td></tr></table>	Mean µg/g	M.O.E. (Aqua Regia Digestion)	109.5	105 (96% recovery)	43.5	44.2 (102% recovery)
Mean µg/g	M.O.E. (Aqua Regia Digestion)						
109.5	105 (96% recovery)						
43.5	44.2 (102% recovery)						

SUMMARY

Limit of detection.	<i>0.02 µg/g.</i> It is likely that greater sensitivities would be achieved by taking larger samples.
Interferences and shortcomings.	There are relatively few compounds that will interfere at the levels normally present in sediment. Of concern are chlorides and oxides of nitrogen but a complete digestion will eliminate most of these problems. Interference from water vapor is eliminated by a water trap and by the use of radiant heat from a tungsten bulb.
Minimum volume of sample.	<i>50 grams</i> of sediment.
Preservation and sample container.	No preservation of sample is possible, thus immediate analysis is a necessity. The sample should be placed in a glass container and contact with metallic substances avoided.
Safety considerations.	Safe handling of acids must be observed, and care taken when samples are acidified because of the possibility of chlorine evolving.

THE DETERMINATION OF TOTAL MERCURY

IN SEDIMENT

1. Introduction

On arrival at the laboratory, the sample is homogenized as much as possible with a glass rod, and duplicate portions of the homogenate are removed manually so as to exclude sticks, large stones (exceeding 2 mm diameter), leaves, etc. These portions are digested in aqua regia and potassium permanganate at a temperature of approximately 100°C. At the same time a portion of sample is weighed into an evaporating dish, dried at 100°C and reweighed. This moisture loss data is used to convert the mercury values found on wet sediment (as it arrived in the laboratory), to values based on dry weight. The digested sample is allowed to cool and hydroxylamine sulfate solution is added to reduce any excess permanganate or manganese dioxide. The sample is then filtered, made up to a fixed volume with distilled water, and immediately prior to purging with air, stannous chloride solution is added to reduce the mercury to its atomic state. (9, 16, 18). The mercury vapor is then purged through the F.A.A.S. system and the resultant absorption is measured spectrophotometrically and presented on a recorder.

2. Interferences and Shortcomings

There are a number of elements or their oxides that can interfere both chemically and/or spectrally with mercury when analyzed using F.A.A.S. Selenium, tellurium, antimony, bismuth and arsenic are reported to interfere chemically by oxidizing the stannous chloride used as reducing agent, but this effect has not been observed in this laboratory. A group of interfering substances that cause problems because of their positive spectral interference are water vapor, NO_2 , Cl_2 , and any volatile organic solvents that absorb light at 253.7 nm. Water vapor is eliminated from the system by the insertion of a coil in an ice bath followed immediately by a chemical water trap prior to the optical cell. As a further precaution, a tungsten element lamp shines on the cell to maintain its temperature about 10°C above ambient, thereby preventing condensation of water vapor on the cell windows.

NO_2 , CO_2 and Cl_2 should not be present after the routine digestion step.

Organic solvents (acetone, chloroform, etc.) should not be used in the laboratory or for cleaning glassware.

3. Apparatus

REFER TO DIAGRAM 1 AND PLATE 1 FOR A PICTORIAL
VIEW OF THE APPARATUS.

- a) Hilger-Watts Atomspek Atomic Absorption Spectrophotometer, or equivalent (*Plate 2*).
- b) Sargent Welch recorder; (*Model SRG*).
- c) Optical cell; 18 cm long with a 1.5 cm diameter and quartz windows.
- d) Drying tube; plastic 11.5 cm x 2.0 cm diameter filled with calcium sulfate. Replace daily.
- e) Hollow cathode lamp; Mercury cathode, A.S.L.
- f) Analytical Balance; single pan (*Mettler*).
- g) Dreschel bottle; 250 ml volume, Quickfit (*BS.2461*), with a 24/29 ground-glass joint.
- h) Mixing Coil; as supplied for Technicon Auto-analyser, (*13 cm long*).
- i) Tungsten element bulb; (*100 watt*), and a retort stand for support; used to heat optical cell.
- j) Flow meter; Gilmont (*size #3*).
- k) Glass dispersion tube; with fritted glass cylinder, 250 mm length, stem diameter 8 mm.
- l) Teflon stopcock; three way, Quickfit 24/29. Used for directing flow of air (1) bypassing sample and (2) through sample, thus aerating it.
- m) Gas bubble counter; (*Canlab HC7778-C*) used as water droplet trap.
- n) Funnels; Fluted, accurate 60°, 65 mm I.D., 150 mm long stem, strong beaded edge.
- o) Beakers, Phillips; Conical, Pyrex with spout, 125 ml volume (*Corning 1080*).
- p) Filter paper; S & S Black ribbon No. 589 (*12.5 cm*).
- q) Hot plate; capable of supplying even heat to 200° (*Lindberg Hevi-duty*).

ALL GLASSWARE AND APPARATUS MUST BE THOROUGHLY
CLEANED PRIOR TO USE.

ALL POLYETHYLENE AND GLASS TUBING SHOULD EITHER BE REPLACED OR RINSED WITH WATER, FOLLOWED BY METHANOL AND BLOWN DRY WITH A STREAM OF AIR.

Cleaning of Apparatus

- a) The optical cell should be cleaned once a week and/or following contamination with excessively high level mercury samples. Rinse cell with (1) water, (2) nitric acid, (3) methanol.

THE CELL SHOULD THEN BE BLOWN DRY WITH AIR.

- b) The drying tube should be refilled with fresh calcium sulfate daily and the tube purged with air to remove dust particles before attaching to the system.
- c) The teflon stopcock should be cleaned at least *once* a week with chromic acid, then water followed by methanol and then air-dried.
- d) The dreschel bottle should be cleaned thoroughly every day or after an extremely high sample, with a cleaning solution containing 1% E.D.T.A. in 20% NaOH. Bottles are usually soaked in this solution for *twelve hours*.

ONLY ONE BOTTLE IS USED FOR STANDARDS AND SAMPLES DURING A RUN BECAUSE THERE IS A "BOTTLE EFFECT" WHICH CAUSES SLIGHT BUT SIGNIFICANT DIFFERENCES IN READINGS FROM BOTTLE TO BOTTLE.

- e) Funnels and Phillips beakers are cleaned immediately after use with (1) soap and water, (2) chromic acid, (3) distilled water.

GLASSWARE MUST BE DRY BEFORE RE-USE.

4. Reagents

ALL REAGENTS SHOULD BE ANALYSED IN THE LABORATORY
AND ONLY THOSE WITH SUFFICIENTLY LOW MERCURY
CONTENT SHOULD BE USED.

- a) Nitric acid; Reagent grade, concentrated. Of several brands investigated, "Baker Analyzed" reagent has been found satisfactory. Reagents other than those mentioned in this section may possess the required degree of purity.
- b) Hydrochloric acid; Reagent grade, concentrated. Mallinckrodt reagent has been found satisfactory.
- c) Potassium Permanganate; Analytical reagent; BDH Analar crystals are suitable.
- d) Hydroxylamine Sulfate; Laboratory reagent. BDH reagent is acceptable.
- e) Stannous Chloride; Analytical reagent. BDH Analar reagent is satisfactory.
- f) Sodium Hydroxide Certified A.C.S., Electrolytic pellets.
- g) Ethylenediaminetetra-Acetic acid; Laboratory reagent, BDH disodium salt is satisfactory.
- h) Mercuric chloride; Reagent grade. As supplied by Mallinckrodt Chemicals.
- i) Methyl mercuric chloride; Reagent grade. Alfa Organics is a satisfactory supplier.
- j) Aqua regia digestion acid

The mixture contains a volumetric ratio of *four* parts distilled water, *three* volumes of concentrated hydrochloric acid and *one* volume of concentrated nitric acid.

CAUTION: EXOTHERMIC REACTION UPON MIXING.

- k) Potassium permanganate solution

This solution should be saturated (60 g/l) at room temperature. The solution should be allowed to stand for at least 48 hours in sunlight before use, to form small amounts of MnO_2 which slowly settles out. This step greatly reduces the risk of mercury contamination from the $KMnO_4$.

4. Reagents

ALL REAGENTS SHOULD BE ANALYSED IN THE LABORATORY
AND ONLY THOSE WITH SUFFICIENTLY LOW MERCURY
CONTENT SHOULD BE USED.

- a) Nitric acid; Reagent grade, concentrated. Of several brands investigated, "Baker Analyzed" reagent has been found satisfactory. Reagents other than those mentioned in this section may possess the required degree of purity.
- b) Hydrochloric acid; Reagent grade, concentrated. Mallinckrodt reagent has been found satisfactory.
- c) Potassium Permanganate; Analytical reagent; BDH Analar crystals are suitable.
- d) Hydroxylamine Sulfate; Laboratory reagent. BDH reagent is acceptable.
- e) Stannous Chloride; Analytical reagent. BDH Analar reagent is satisfactory.
- f) Sodium Hydroxide Certified A.C.S., Electrolytic pellets.
- g) Ethylenediaminetetra-Acetic acid; Laboratory reagent, BDH disodium salt is satisfactory.
- h) Mercuric chloride; Reagent grade. As supplied by Mallinckrodt Chemicals.
- i) Methyl mercuric chloride; Reagent grade. Alfa Organics is a satisfactory supplier.
- j) Aqua regia digestion acid

The mixture contains a volumetric ratio of *four* parts distilled water, *three* volumes of concentrated hydrochloric acid and *one* volume of concentrated nitric acid.

CAUTION: EXOTHERMIC REACTION UPON MIXING.

- k) Potassium permanganate solution

This solution should be saturated (60 g/l) at room temperature. The solution should be allowed to stand for at least 48 hours in sunlight before use, to form small amounts of MnO_2 which slowly settles out. This step greatly reduces the risk of mercury contamination from the $KMnO_4$.

l) Hydroxylamine sulfate solution

The solution (20% w/v) is prepared by dissolving 200 grams of reagent grade (B.D.H.) hydroxylamine sulfate in one litre of distilled water. This solution is used to reduce excess permanganate after digestion.

CAUTION: THIS SUBSTANCE IS CARCINOGENIC: AVOID CONTACT WITH THE SKIN.

m) Stannous Chloride solution

The solution (20% w/v) is prepared by dissolving 400 grams of reagent grade stannous chloride (B.D.H.) in two litres of concentrated hydrochloric acid. This solution is used to reduce the mercury to its atomic form (Hg^0).

n) Mercuric chloride stock solution

Prepared by dissolving 1.358 grams in one litre of distilled water containing 10 mls of concentrated nitric acid. Concentration: 1000 ug/ml.

o) Methyl mercuric chloride stock solution

Prepared by dissolving 0.6258 grams of methyl mercuric chloride in 1000 mls of methanol (reagent grade). Concentration: 500 ug/ml.

p) Mercury standards

Working solutions containing 1 ug mercury per ml should be made up by making successive dilutions from the stock solution in distilled water. New working solutions should be made up weekly and the concentration of mercury in these should be within 1% of the old ones.

5. Procedure

- a) Decant excess liquid from the sample bottle and homogenize the sample with a glass rod. Manually remove portions of the homogenate with a scoopula, taking care to exclude sticks, large stones (exceeding 2 mm in diameter), leaves and other extraneous material.
- b) Weigh duplicate portions of 2 to 4 grams of the selected homogenate into tared 125 ml Phillips beakers using a single pan analytical balance, to four significant figures. Record the weight of the sample.
- c) At the same time a portion of homogenate, approximately ten grams, is weighed into a tared evaporating dish. The weight of sample and dish are recorded and the sample dried at 110°C for a minimum of three hours. After drying to constant weight, the sample and dish

are re-weighed and a factor is derived that indicates the relationship of dried sample compared to wet sample. This factor is eventually applied to the weight of the sample taken for analysis to obtain the mercury concentration on a dry weight basis.

- d) The samples are prepared as a series of blanks, standards and controls called a "run".

A "run" of samples consists of (1) *two* reagent blanks, (2) *two* 1 μg inorganic standards, (3) *two* 1 μg organic standards, (4) *two* control sediments, (5) *one* inorganic mercury spike*, (6) *one* organic mercury spike*. The control sediment is a homogenous sample that has been previously analyzed at least *twenty* times.

*Spike samples are arbitrarily selected sediments within the run that are spiked with known amounts of inorganic or organic mercury standards (0.5 μg Hg usually) to determine the percent recovery of these mercury compounds.

- e) To the sample add a few anti-bumping granules (B.D.H.) and 10 ml of the aqua regia digestion acid.
- f) Place the digestate on a hot plate at medium heat, swirl the samples to prevent bumping and foaming, and heat for *two minutes*.

DO NOT ALLOW SAMPLES TO BOIL. REMOVE SAMPLES FROM HOT PLATE AND ALLOW TO COOL.

- g) Add 50 ml of distilled water, washing down the beaker sides. Add 15 ml of saturated KMnO_4 solution, reheat the samples to incipient boiling and allow to digest for *thirty minutes*. Care should be taken that bumping of the samples does not occur. Remove samples from hotplate and allow to cool.
- h) Add 3 ml of (20% w/v) hydroxylamine sulfate to reduce the excess permanganate and manganese dioxide.

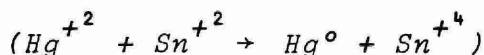
THIS STEP SHOULD NOT BE COMPLETED UNTIL IT HAS BEEN ASCERTAINED THAT THE SAMPLES CAN BE ANALYZED WITHIN THE NEXT HOUR. ONCE THE STEP IS PERFORMED, SAMPLES MUST BE ANALYZED WITHIN THE NEXT HOUR.

- i) Filter the sample through S & S Black Ribbon No. 589 (12.5 cm) filter paper, into 100 ml tubes and make up to volume with distilled water.

Instrument Operation

See "The Determination of Total Mercury in Biological Material", under "Procedure".

- a) Decant the sample into a dreschel bottle and add 3.0 ml of (20% w/v) stannous chloride solution to reduce the mercury.



- b) Immediately attach the dreschel bottle to the apparatus and aerate the sample.
- c) After maximum absorbance has been attained, flush the system by aerating a bottle containing distilled water only, until the original baseline is reached on the recorder.

Special Precautions

1. Periodically the instrument should be checked for drift from zero position.
2. If any changes to the system are made during the run, new standards should be analyzed to determine variations in sensitivity.
3. Moisture content in the water trap should be periodically checked. Empty as needed to prevent bubbling.

6. Calculation and Reporting

Formula: $\mu g/g$ mercury as Hg = $\frac{x - b}{(a - b) n \times d}$

x = peak height of sample

a = average height of digested standard

b = blank peak height

n = weight in gram

d = drying factor = $\frac{\text{weight of dried sample}}{\text{weight of wet sample}}$

Reporting

Range in $\mu g/g$

Reported in $\mu g/g$

<0.02

<0.02

0.02 to 0.09

1 significant figure

0.10 to 0.99

2 significant figures

1.0 to 99

2 significant figures

THE DETERMINATION OF TOTAL MERCURY
IN AQUEOUS SOLUTIONS
METHOD A

SUMMARY

Substance determined.	Total mercury at concentrations less than 10 ng/ml in aqueous samples.
Application of method.	Experience of analyst will determine type of sample to be analyzed using this procedure. Results indicate the level of total mercury in the sample.
Principle of method.	Mercury in the sample is converted to the inorganic form by digestion procedure. The inorganic mercury is reduced in aqueous solution with stannous sulfate. A stream of air is bubbled through the vessel containing the reduced mercury. The air stream carries the mercury vapor into a flow-through absorption cell positioned between a 253.7 nm wavelength light source and a detector. The amount of absorption is proportional to the concentration of mercury in the sample.
Time required for analysis.	A single analysis takes about <i>four hours</i> . However, <i>twenty</i> analyses per day can be completed when the samples are tested in batches.
Range of application.	0.05 ng/ml to 10 ng/ml.
Precision.	± 0.03 ng/ml on a sample containing 0.2 ng/ml. ± 0.14 ng/ml on a sample containing 8.8 ng/ml. (Part of an E.P.A. method evaluation).
Accuracy.	Within the boundaries set by the precision.
Limit of detection.	0.05 ng/ml. The detection limit is based on the blank value for the particular series of samples being analyzed. In this test, twice the value of the blank is considered the detection limit.
Interferences and shortcomings.	Water vapor can condense on the windows of the absorption cell, causing absorption of radiation at the 253.7 nm resonance line of mercury. However, two systems can be used to remove the water vapour interference. a) A bubbler immersed in an ice bath will condense

SUMMARY

moisture passing through the system, resulting in a low constant water vapor level.

b) a moisture trap containing magnesium perchlorate will remove moisture from the system. Hydrogen sulfide can interfere, but is eliminated by potassium permanganate. Elevated chloride concentrations are removed by the sulfuric, nitric acid and permanganate oxidation step.

Aromatic hydrocarbons, HCl gas, CO_2 and other gases that are capable of absorbing light at 253.7 nm will interfere, but these are generally removed by adequate sample preparation.

Minimum volume
of sample.

At least 250 ml s are required if duplicate analyses are to be performed.

Preservation and
sample container

For total mercury, Pyrex glass bottles should be used and the bottle caps should be lined with Teflon. The samples must be preserved with enough concentrated nitric acid (Baker Analyzed only) to bring the pH below 1, and enough saturated potassium permanganate solution to maintain a purple colour.

Safety
considerations.

Samples suspected to be high in halides should be acidified and digested in a fume hood for the protection of the analyst.

THE DETERMINATION OF TOTAL MERCURY
IN AQUEOUS SOLUTIONS
METHOD A

1. Introduction

A mixture of sulfuric and nitric acids is added in order to break down organics in the sample; potassium permanganate and potassium persulfate are added to further oxidize the sample. A solution containing stannous sulfate, hydroxylamine sulfate and sodium chloride is used to reduce the mercury present to its atomic form. (22).

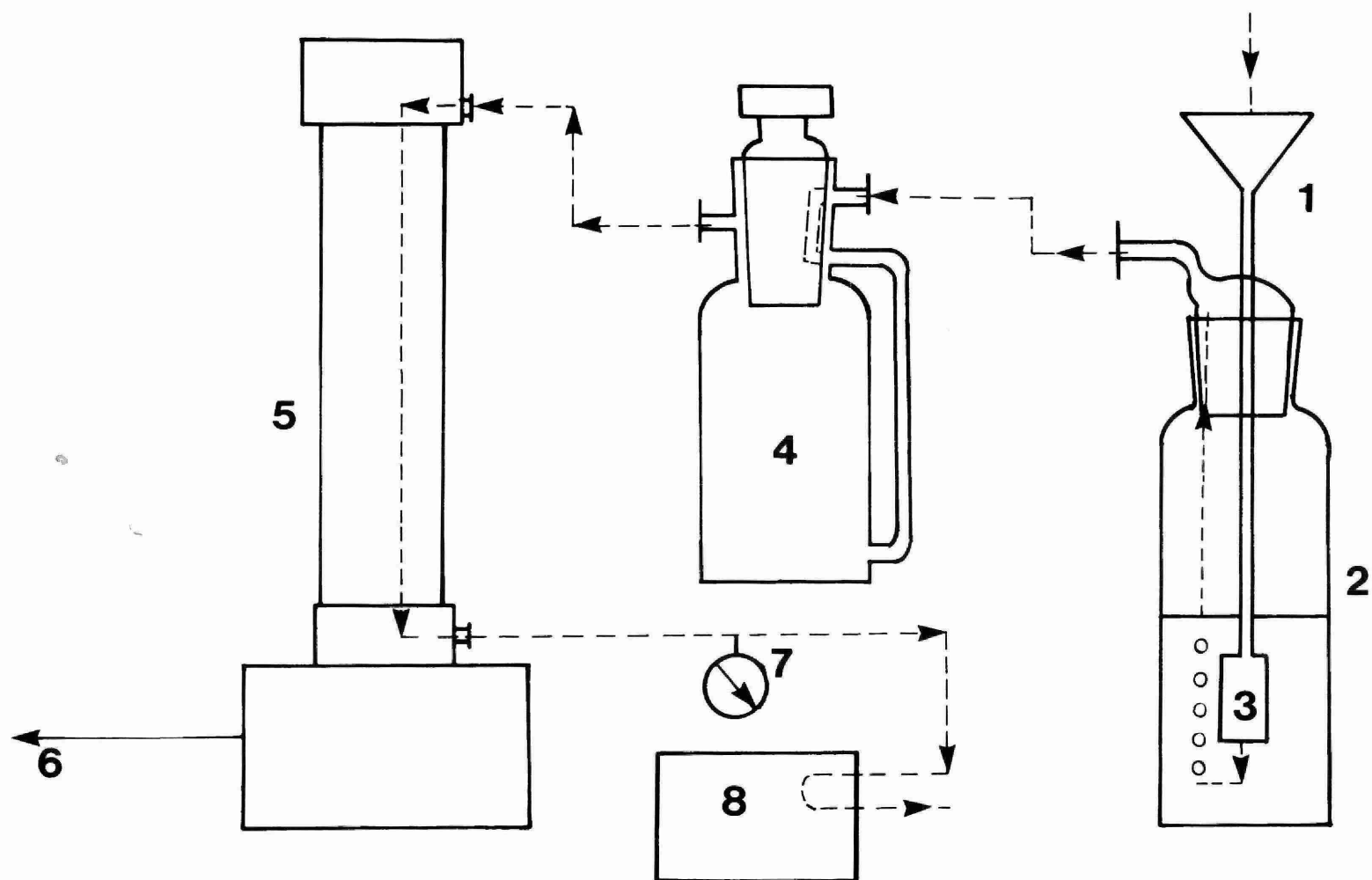
After reduction of mercury, a stream of air is bubbled through the solution by means of a vacuum system. The mercury vapor is carried into a flow-through absorption cell positioned between a source of 253.7 nm wavelength light and a detector, which measures the degree of attenuation of the source light. This absorption is displayed on a recorder and the tracings are compared with those of known standards. The method described has application in the analysis of potable and surface waters, and industrial and sewage treatment plant effluents which contain extremely low levels of mercury.

2. Interferences and Shortcomings

Water vapor at a sufficiently high concentration in the system can condense on the windows of the absorption cell, causing absorption of the radiation at the 253.7 nm resonance line of mercury. This problem can be overcome by removing a sufficient amount of the water vapor so that condensation cannot occur. The insertion of a bubbler submerged in an ice bath reduces the vapour level.

Other interfering substances are hydrogen sulfide, aromatic hydrocarbons, carbon dioxide and other gases capable of absorbing light at 253.7 nm, but these are generally removed by an adequate sample preparation.

Samples suspected of containing high levels of chloride should be handled with extreme care, since toxic chlorine gas can evolve upon addition of sulfuric, nitric acid and potassium permanganate during the determination. The suspect sample should be placed in a fume hood and a portion of the sample should be neutralized to pH 7. This aliquot is then tested with starch and iodide test paper, which will turn blue if chlorine is being evolved. Samples which test positively should be analyzed by Method B.



1. Funnel & Dreschel bottle head
 3. Glass frit
 5. L.D.C. Mercury monitor
 7. Flow meter

2. 300ml Dreschel bottle
 4. Magnesium perchlorate trap
 6. L.D.C. Output to monitor & readout
 8. Vacuum pump

Diagram 6

Water aeration & mercury determination apparatus

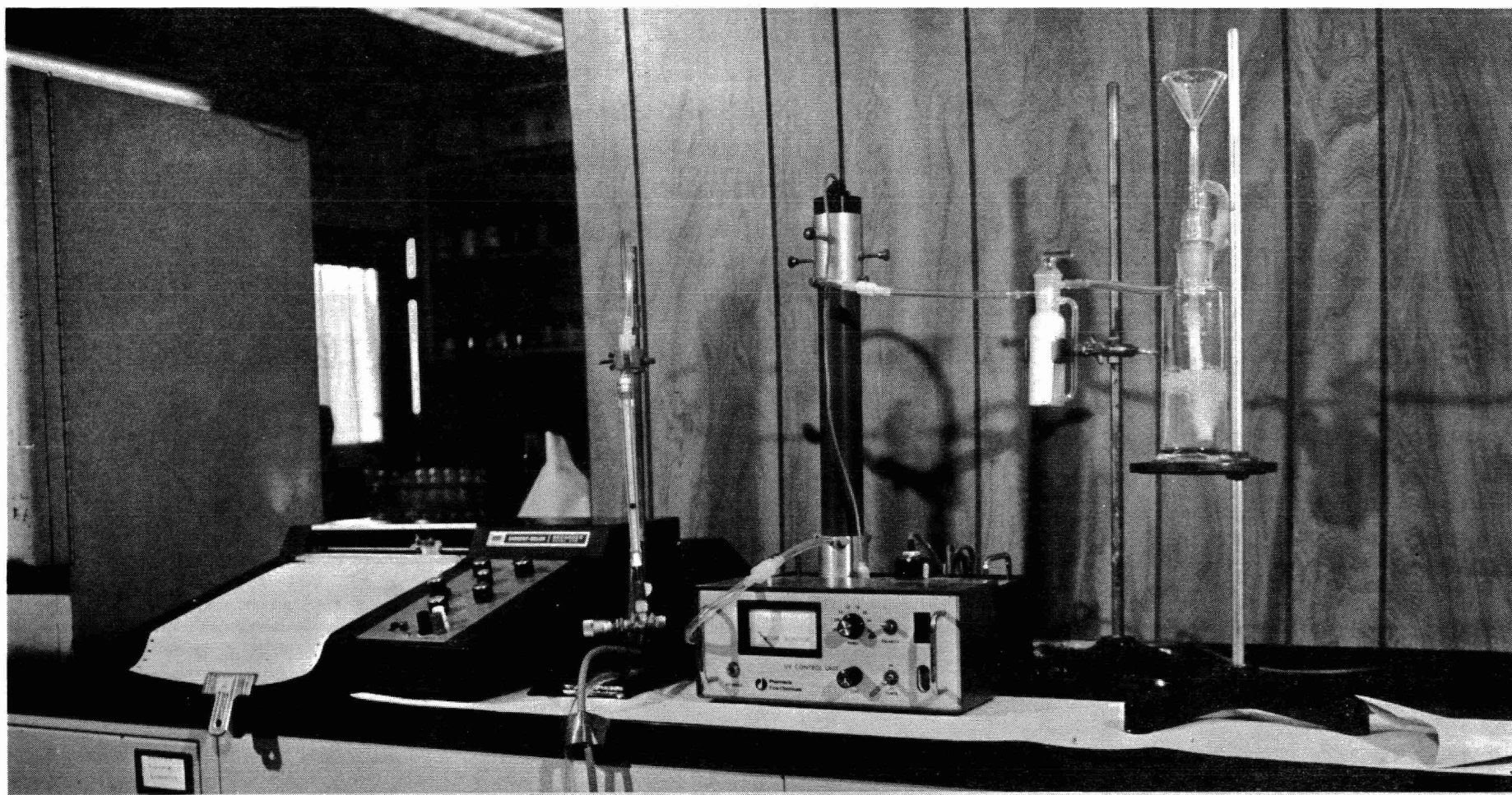


Plate 5 Water aeration & mercury determination apparatus

3. Apparatus

PLEASE REFER TO DIAGRAM 6 AND PLATE 5 FOR A
VISUAL DISPLAY.

- a) Water bath; capable of maintaining a temperature of 85-90°C.
- b) LDC Mercury Monitor; model 1265, supplied by Pharmacia Ltd.
- c) Air-Vacuum pump; Millipore (Cat. #XX6000,000,000), capable of delivering air flow of 3 litre/min.
- d) Recorder; Sargent-Welch, model S.R.G.
- e) Flowmeter; Gilmont (size #3).
- f) Glass dispersion tube; with fritted glass cylinder, 250 mm length, stem diameter 8 mm.
- g) Bottles; Special Bacteriological, 16 oz. square with Bakelite cap and teflon liners. Bottles should be cleaned in chromic acid, 1:1 HNO₃ and rinsed in tap water, then in distilled water.
- h) Drying tube; containing magnesium perchlorate, should be changed after every twenty samples.

4. Reagents

ALL REAGENTS SHOULD BE ANALYSED IN THE LABORATORY
AND ONLY THOSE WITH A SUFFICIENTLY LOW MERCURY
CONTENT SHOULD BE USED.

- a) Sulfuric acid; concentrated, reagent grade. Must contain no measurable mercury content (Aristar recommended). Reagents other than those recommended in this section may possess the required degree of purity.
- b) Nitric acid; concentrated reagent grade. Must contain no measurable mercury content (Baker Analyzed recommended).
- c) Potassium Permanganate; Analytical reagent, Analar (BDH).
- d) Potassium Persulfate; Analytical reagent, Analar (BDH).
- e) Sodium Chloride; Analytical reagent, Analar (BDH).
- f) Stannous Sulfate; Analytical reagent, Analar (BDH).
- g) Hydroxylamine Sulfate; Laboratory reagent (BDH).
- h) Magnesium Perchlorate; Laboratory reagent (BDH).
- i) Mercuric Chloride; Reagent grade, Mallinckrodt Chemicals.
- j) Methyl Mercuric Chloride; Reagent Grade, Alfa Organics.
- k) Sodium chloride-hydroxylamine sulfate solution

Twelve grams of NaCl and twelve grams of hydroxylamine sulfate made up to 100 mls with distilled water.

- l) Stannous sulfate solution; (10% w/v)

Twenty five grams of stannous sulfate dissolved in 0.5N sulfuric acid to a final volume of 250 mls.

- m) Potassium permanganate solution; (saturated)

Dissolve 60 grams of Potassium Permanganate in 1 litre of distilled water. Allow solution to stand in sunlight for at least 48 hours before use, to form small amounts of MnO_2 , which slowly settles out. This step greatly reduces the risk of mercury contamination from the $KMnO_4$.

- n) Potassium persulfate solution (5% w/v)

Dissolve 5 grams of potassium persulfate in 100 mls of distilled water.

o) Mercuric chloride stock solution

Prepared by dissolving 1.358 grams HgCl_2 in 1 litre of distilled water containing 10 mls of concentrated nitric acid. Concentration: 1000 ug/ml.

p) Methyl mercuric chloride stock solution

Prepared by dissolving 0.6258 grams of methyl mercuric chloride in 1000 mls of methanol. (Reagent grade). Concentration: 500 ug/ml.

q) Mercury standards

Prepared by making successive dilutions of the stock mercury solutions to obtain working standard solutions containing 10 ng/ml of mercury in the inorganic and organic forms.

5. Procedure

- a) Transfer 1.0, 2.0, 4.0 ml aliquots of the 10 ng/ml mercury working solutions (both inorganic and organic) to the bacti bottles. Make up to 100 mls with distilled water and mix thoroughly. For samples 100 ml aliquots are taken in duplicate. If smaller aliquots are used, they are made up to a final volume of 100 mls with distilled water.
- b) Add 5.0 mls of concentrated sulfuric acid and 2.5 mls of concentrated nitric acid to each bottle. The addition of sulfuric acid to the sample generates heat, but with the stated amount of acid and 100 ml of aliquot the temperature rise is only about 13°C and no loss of mercury should occur.
- c) Add 1 ml of the saturated potassium permanganate solution to each bottle and allow to stand at least 15 minutes. Add 2 mls of the potassium persulfate solution to each bottle and mix well.
- d) Heat samples (with caps loosened) for 2 hours at 85°C to 90°C in the water bath.
- e) Remove samples from water bath and cool to room temperature.
- f) Add 2 mls of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate present in the samples.

- g) Treating each bottle individually, transfer the sample to a dreschel bottle, add 5 ml's of stannous sulfate solution and aerate the sample immediately by connecting to the vacuum FAAS system.
- h) The atomic mercury (Hg^0) absorbs some of the light emitted by the UV lamp, and this absorption is displayed as a peak on a recorder. After the absorbance reaches a maximum and the recorder pen returns close to the original base line (zero absorbance), the dreschel bottle is removed and the system is allowed to come to equilibrium.
- i) A standard curve is plotted from the absorption measurements of the standards that are run in parallel with the samples. The curve obeys Beer's Law up to 100 ng and the majority of water samples are either in this range or can be diluted so as to fall within the workable portion of the curve.

6. Calculation and reporting

Formula:

$$\mu g/ml \text{ of mercury in sample} = \frac{(a-c) \times d}{(b-c) \times e}$$

a = peak height of sample

b = peak height of standard

c = peak height of blank

d = standard in μg

e = volume of sample taken (ml).

Reporting:

Range in $\mu g/ml$	Reported in $\mu g/ml$
< 0.05	< 0.05
0.05 to 0.09	1 significant figure
0.10 to 9.9	2 significant figures
10 to 100	2 significant figures

7. Precision and Accuracy

A series of eight samples received from E.P.A. ranging from 0.2 to 10 $\mu g/ml$ were analyzed by this method and the average accuracy was found to be 99.3%. At the 0.4 $\mu g/ml$ level the precision was 3.6%.

THE DETERMINATION OF TOTAL MERCURY
IN AQUEOUS SOLUTIONS

METHOD B

SUMMARY

Substance determined.	Total mercury in aqueous samples.
Application of method.	As in Method A the experience of the analyst and sampler will determine the method to be applied.
Principle of method.	Mercury in the sample is converted to the inorganic form by the digestion procedure. The inorganic mercury (Hg^{+2}) is reduced in aqueous solution, by hydroxylamine sulfate and stannous chloride solutions, to its atomic form. An air stream carries the mercury vapor into an optical cell, where the absorption is determined by the monochromator and measuring circuits of an A.A.S. and displayed on a recorder. The peak traced by the recorder is compared to standards previously run and the concentration of mercury is calculated based on peak heights and aliquot factors.
Time required for analysis.	A single analysis takes about <i>four hours</i> . However, <i>twenty</i> analyses per day can be completed when the samples are tested in batches.
Range of application.	0.2 to 1000 ng/ml.
Precision	± 0.04 ng/ml on a sample containing 0.27 ng/ml. ± 0.08 ng/ml on a sample containing 9.6 ng/ml. (part of an E.P.A. method evaluation).
Accuracy	Within limits defined by precision data.
Limit of detection.	0.2 ng/ml.
Interferences and shortcomings.	Water vapor will absorb radiation from the hollow cathode lamp at 253.7 nm. A constant water vapor trap and a chemical water trap, combined with heating the optical cell above ambient temperature, will alleviate the problem. Most of the common interferences (CO_2 and aromatic hydrocarbons) are removed by the oxidizing acid digestion. The

SUMMARY

method is limited by the high blank values, which are unavoidable because of the large volumes of reagents required.

Minimum volume
of sample.

At least 250 *mls* of sample if duplicate analysis is to be performed.

Preservation
and sample
container.

For total mercury, Pyrex glass bottles should be used, with Teflon caps.

At present, samples are preserved with 1 *ml* of concentrated nitric acid (Baker Analyzed only) *per litre* of sample and enough $KMnO_4$ solution to maintain a purple colour.

Safety
considerations.

Samples high in halides (chlorides) should be acidified and digested in a fume hood for the protection of the analyst.

THE DETERMINATION OF TOTAL MERCURY

IN AQUEOUS SOLUTIONS

METHOD B

1. Introduction

The sample is digested on a hot plate with a mixture of sulfuric acid (1:1) and potassium permanganate (saturated) to convert the mercury present to the inorganic form. The digestate is then treated with hydroxylamine sulfate solution (20% w/v) to reduce the excess potassium permanganate. After reduction with stannous chloride (20% w/v) the mercury is brought to its atomic form and the sample is aerated immediately. The mercury vapor is purged through the F.A.A.S. system and an absorption peak is measured by a recorder connected to the A.A.S. readout. The sample absorption tracings are then compared to the standard peaks and the sample's mercury concentration is calculated. (16, 17, 18).

2. Interferences and shortcomings

Water vapor can absorb radiation at the 253.7 nm resonance line of mercury. The insertion of a constant water vapor system and chemical water trap, combined with heating the optical cell, eliminates this problem.

Other interfering substances are hydrogen sulfide, aromatic hydrocarbons, carbon dioxide, chlorine and other gases capable of absorbing light at 253.7 nm. These can be removed by digestion with oxidizing acids.

3. Apparatus

PLEASE REFER TO DIAGRAM 1 AND PLATE 1 FOR A
VISUAL DISPLAY OF THE APPARATUS.

- a) Hilger-Watts Atomspek Atomic Absorption Spectrophotometer, or equivalent, (plate 2).
- b) Recorder; Sargent-Welch, model S.R.G.
- c) Hollow Cathode Lamp; Mercury A.S.L.
- d) Optical cell; 18 cm long with a 1.5 cm diameter and quartz windows.
- e) Drying tube; plastic 11.5 cm x 2.0 cm diameter filled with calcium sulfate. Replace daily.
- f) Dreschel bottle; 250 ml volume; Quickfit (BS2461) with a 24/29 ground joint.

- g) Beaker, Griffin; low form, with spout, 250 ml, graduated.
- h) Cover, Beaker; speedivap for 250 ml beakers. (3.1/2 inch diameter).
- i) Mixing coil; as supplied for Technicon Auto Analyzer (13 cm long).
- j) Tungsten element bulb; (100 watt), and a retort stand for support; used to heat optical cell.
- k) Flow meter; Gilmont (size #3).
- l) Gas dispersion tube; with fritted glass cylinder, 250 mm length, stem diameter 8 mm.
- m) Teflon stopcock; three way, Quickfit 24/29. Used for directing flow of air (1) bypassing sample and (2) through sample.
- n) Gas bubble counter; (Canlab #C7778-C) used as a water droplet trap.

*ALL GLASSWARE AND APPARATUS MUST BE THOROUGHLY
CLEANED PRIOR TO USE.*

Cleaning of Apparatus

See "The Determination of Total Mercury in Biological Material".

4. Reagents

ALL REAGENTS SHOULD BE ANALYZED IN THE LABORATORY AND ONLY THOSE WITH A SUFFICIENTLY LOW MERCURY CONTENT SHOULD BE USED.

- a) Sulfuric acid; Reagent grade, containing less than 1 ppb mercury (Baker or MacArthur reagent recommended), concentrated. Reagents other than those recommended in this section may possess the required degree of purity.
- b) Potassium Permanganate; Analytical reagent, Analar (BDH) crystals have been found satisfactory.
- c) Hydroxylamine sulfate; Laboratory reagent (BDH).
- d) Stannous Chloride; Laboratory reagent, Analar (BDH).
- e) Hydroxylamine sulfate solution

The solution (20% w/v) is prepared by dissolving 200 grams of reagent grade (BDH) hydroxylamine sulfate in one litre of distilled water. This solution is used to reduce the excess permanganate after digestion.

- f) Stannous Chloride solution

The solution (20% w/v) is prepared by dissolving 400 grams of reagent grade stannous chloride (BDH) in two litres of concentrated hydrochloric acid. This solution is used to reduce the mercury to its atomic form (Hg^0).

- g) Digestion Acid

The mixture contains a volumetric ratio of one part sulfuric (concentrated) acid to one part distilled water.

- h) Mercury stock solution

Prepared by dissolving 1.354 grams of mercuric chloride in approximately 750 mls of distilled water, then adding 10 mls of concentrated nitric acid and adjusting the volume to 1 litre with distilled water. Final concentration is 1000 ug/ml.

- i) Methyl mercury stock solution

Prepared by dissolving 0.6258 grams of methyl mercuric chloride in 1000 mls of methanol (reagent grade). Concentration is 500 ug/ml.

j) Mercury standards

Prepared by making successive dilutions of the stock mercury solution to obtain a working standard containing 100 ng/ml. This solution should be made up daily.

k) Potassium Permanganate solution

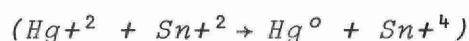
The solution should be saturated (60 g/l) at room temperature. The solution should be allowed to stand for at least 48 hours in sunlight before use, to form small amounts of MnO_2 which slowly settles out. This step greatly reduces the risk of mercury contamination from $KMnO_4$.

5. Procedure

- a) Sample bottle should first be well shaken and all subsequent operations performed in a fumehood.
- b) Transfer 100 ml of sample to acid washed 250 ml Pyrex beakers. The pH of the sample should be checked; if the sample is highly caustic it should be neutralized with diluted sulfuric acid.
- c) Add 15 mls of saturated potassium permanganate solution. If purple colour fades, add more potassium permanganate to maintain colour.
- d) Add 10 ml of 1:1 (v/v) sulfuric acid.
- e) Transfer beakers with speedivap covers to a hot plate at medium heat and bring sample to an incipient boil. Maintain this temperature and reduce sample volume to 50 mls.
- f) Allow samples to cool to room temperature.
- g) Reduce the manganese oxide and excess potassium permanganate with 1 ml of 20% hydroxylamine sulfate solution.

AFTER THIS REDUCTION STEP THE ANALYSIS SHOULD TAKE PLACE WITHIN THE NEXT HOUR.

- h) The sample is then transferred to volumetric glassware and made up to 100 mls with distilled water.
- i) The sample is then transferred to a Dreschel bottle and 30 ml of 20% (w/v) stannous chloride solution is added to reduce the mercury.



- j) Immediately attach the Dreschel bottle to the apparatus and aerate the sample.

- k) Operation of the A.A.S. equipment and daily warm-up procedure are provided in *The Determination of Total Mercury in Biological Material*, under "Procedure".
- l) After maximum absorbance has been attained, flush the system by aerating a bottle containing distilled water only, until the original baseline is reached on the recorder.

Special Precautions

- 1) Periodically the instrument should be checked for drift from zero position.
- 2) If any changes to the system are made during the run, new standards should be analyzed to determine variations in sensitivity.
- 3) Moisture content in the water trap should be periodically checked, and the trap should be emptied as needed to prevent bubbling.

6. Calculation and Reporting

Several standards and blanks are carried through the procedure and are used to prepare a calibration curve. This standard curve can be used to calculate mercury concentrations in samples, or the following formula may be used.

Formula:

$$\text{ng/ml of mercury in sample} = \frac{(a-c)}{(b-c)} \times d$$

a = peak height of sample,

b = peak height of standard,

c = peak height of blank,

d = standard in ng,

e = volume of sample taken (ml).

Reporting:

Range in ng/ml	Reported in ng/ml
<0.2	<0.2
0.20 to 9.9	2 significant figures
10.0 to 99.9	3 significant figures

THE DETERMINATION OF METHYL MERCURY
IN FISH MUSCLE BY GAS CHROMATOGRAPHY

SUMMARY

Substance determined.	Methyl mercuric bromide, CH_3HgBr .
Interpretation of Results	Results are reported as $\mu\text{g/g}$ mercury as CH_3HgBr . The methyl mercury results are calculated without any correction factor. However, studies using spiked fish tissue imply that only $89\pm 5\%$ of the methyl mercury is recovered in the test used.
Principle of Method.	The CH_3Hg^+ is released from its muscle protein complex by the addition of CuSO_4 and NaBr in acid, then extracted as the bromide into toluene. Cleanup is achieved by extraction of the methyl mercury as its thiosulfate complex into aqueous $\text{Na}_2\text{S}_2\text{O}_3$. This complex is broken by the addition of CuBr_2 , and the CH_3HgBr formed is extracted into benzene and analyzed quantitatively on a gas chromatograph.
Time required for analysis.	One and a half days are required for a single analysis. However, twenty to thirty fish are usually analyzed in a batch, which requires the same length of time.
Range of application.	$0.01 \mu\text{g/g}$ of Hg as methyl mercuric bromide is detectable on 1 g of fish muscle. Very high concentrations may be analyzed by dilution techniques.
Precision.	$\pm 10\%$ relative on concentrations over $0.1 \mu\text{g/g}$.
Accuracy.	Studies using spiked fish tissue imply that only $89\pm 5\%$ of the methyl mercury is recovered in the test used.
Limit of detection.	For samples of 1 g of muscle, $0.01 \mu\text{g/g}$ is the limit of detection, but this can be lowered by taking a larger sample.
Interferences and shortcomings.	There are no known interferences to this method. Prolonged exposure to light during sample work-up can lead to low and variable results due to the photochemical breakdown of the methyl mercury by light.

SUMMARY

Minimum volume of sample.	For concentrations down to $0.1 \mu\text{g/g}$, 1 g of muscle is required.
Preservation and sample container.	Muscle samples should be frozen and protected from light except during processing.

THE DETERMINATION OF METHYL MERCURY
IN FISH MUSCLE BY GAS CHROMATOGRAPHY

1. Introduction

The methyl mercury is released from its protein complex in the muscle by the addition of cupric sulfate and an acidic solution of sodium bromide. The methyl mercury is extracted into toluene as methyl mercuric bromide. Interferences are removed by the extraction of the methyl mercury as its thiosulphate complex into an aqueous sodium thiosulphate solution. This complex is broken down by the addition of cupric bromide, and the methyl mercuric bromide is extracted into benzene and analyzed quantitatively by gas chromatography. (23, 24, 25, 26).

2. Interferences and shortcomings

There are no known interferences in this method.

3. Apparatus

- a) Analytical balance; capable of weighing to 0.1 mg.
- b) Actinic 25 ml culture tubes with Teflon lined screw caps. (30).
- c) Graduated actinic 10 ml centrifuge tubes with teflon lined screw caps. (30).
- d) Actinic 10 ml culture tubes with Teflon lined screw caps. (30).
- e) Centrifuge capable of accepting 50 ml culture tubes at 2,000 rpm.
- f) Clinical centrifuge for 10 ml centrifuge tubes.
- g) Disposable Pasteur pipettes.
- h) Polytron Homogenizer.
- i) Assorted pipettes.
- j) Recorder with 1 mv full scale deflection.
- k) Gas chromatograph with a supply of ultra-pure nitrogen and a tritium electron capture detector.

Gas Chromatograph Conditions:

Column: 6' x 1/8" i.d. Pyrex glass column packed
 with 5% carbowax 20M on Varaport 30
 (100-120 mesh).

Gas Flow; N₂ (ultra-pure); 45 ml/min.

Injector Temp: 200°C.

Oven Temp: 180°C.

Detector: e.c. (250 mC: ³H); 210°C.

4. Reagents

a) Benzene - distilled in glass.

b) Cupric Bromide Solution

22.34 g of analytical reagent grade $CuBr_2$ is dissolved in 100 ml distilled water and extracted with 2 x 150 ml benzene. This solution is prepared daily.

c) Cupric Sulphate Solution

250 g of analytical reagent grade $CuSO_4 \cdot 5H_2O$ is dissolved in 1 litre of distilled water. This solution is prepared weekly and is stored in the dark.

d) Sodium Bromide Solution

110 ml of concentrated reagent grade H_2SO_4 is added to 110 ml of distilled water slowly. 310 gm of reagent grade $NaBr$ is dissolved in 700 ml of distilled water. The solutions are combined and made to 1 litre with distilled water. This solution is made weekly.

e) Sodium Thiosulphate Solution

12.4 g of reagent grade $Na_2S_2O_3 \cdot 5H_2O$ is dissolved in 1 litre of distilled water. 100 mg anhydrous Na_2CO_3 is added. This solution is diluted 1:10 with distilled water as required. The stock solution is made bi-weekly.

f) Toluene - Reagent Grade.

g) Water

All water used in the procedure is prepared by extracting 1 litre of distilled water with 2 x 100 ml of benzene.

5. Procedure

Physical Preparation

See "The Determination of Total Mercury in Biological Material".

a) Approximately 1 g of homogenized fish muscle is accurately weighed into a numbered 25 ml screw-capped actinic culture tube, and 5 ml of extracted, distilled water is added.

- b) The fish samples in the water are homogenized for 1 minute on the Polytron homogenizer. The homogenizer probe is washed down into the tube with 5 ml of distilled water. The probe is cleaned between samples with distilled water.
- c) To each tube, 1 ml of the cupric sulphate solution, and 5 ml of the sodium bromide solution are added. The tubes are capped and allowed to stand overnight in the dark.
- d) Ten ml of toluene is added to each tube, and the tubes are capped and agitated manually for two minutes.
- e) The tubes are balanced and centrifuged for five minutes at 2,000 rpm.
- f) Five ml of the toluene layer is transferred by Pasteur pipette and a syringe into a 10 ml graduated actinic centrifuge tube. The pipette is discarded after each sample.
- g) To the toluene extract, two mls of the sodium thiosulphate solution is added and the tubes are capped, then shaken manually for two minutes. After shaking, the tubes are centrifuged.
- h) The thiosulphate layer is transferred quantitatively by Pasteur pipette syringe to a 10 ml actinic screw-cap culture tube. The pipette is discarded after each sample.
- i) To the thiosulphate layer, 2 ml of the cupric bromide solution and 1.0 ml benzene is added. The tubes are capped and manually agitated for one minute.
- j) The benzene layer is transferred by Pasteur pipette syringe to an actinic 10 ml screw-capped tube and stored, tightly capped, in a dark refrigerator prior to gas chromatographic analysis.

Special Precautions

ANALYSIS OF THE FINAL BENZENE EXTRACTS SHOULD BE DONE AS QUICKLY AS POSSIBLE. THE ANALYSIS SHOULD BE REPEATED IF THE EXTRACTS HAVE BEEN LEFT STANDING MORE THAN THREE DAYS.

All glassware should be cleaned in the following way:

- i) Wash glassware thoroughly with alcoholic KOH (100 g KOH in 1 litre 20% C_2H_5OH).

- ii) Rinse well with tap water.
- iii) Rinse twice with an aqueous $0.1M Na_2S_2O_3$ solution.
- iv) Rinse twice with distilled water.
- v) Rinse with acetone and dry in air.

6. Calculation and Reporting

$$\mu g/g \text{ Hg as } CH_2HgBr = \frac{a \times b \times 2}{c \times d \times 10^3}$$

where

a = the dilution factor of the benzene layer.

b = the number of nanograms corresponding to the peak height on the chromatogram.

c = the number of microlitres of the benzene layer injected into the gas chromatograph.

d = the weight of fish taken (g).

The factor of 2 arises since only half of the toluene layer is taken. The factor of 10^3 in the denominator converts the result in ng/g to $\mu g/g$.

Except for results below 0.01 mg/l , all results are reported to *three significant figures*.

A recovery factor is not included in this equation. Recovery studies have shown that only 89% of the methyl mercury is recovered from spiked fish muscle samples or spiked aqueous samples. This recovery factor agrees well with the theoretical maximum of 90% (34, 35), and with experimental values found by Westoo (23) and Nishi (35). Since a recovery factor is not used in the calculations, an explanatory note is included with all reported results:

"The methyl mercury results are calculated without a recovery factor. However, studies using spiked fish tissue imply that only $89\% \pm 5\%$ of the methyl mercury is recovered. The deviation of duplicate results is within $\pm 10\%$ ".

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